PART IV
ANALYSIS OF THE DATA

Discussion

"You can not connect the dots looking forward. You can only connect them, looking backward. You have to trust that the dots will somehow connect"

"Only paranoids survive!"

~ Steve Jobs ~
IV: ANALYSIS OF DATA:

8. Discussion

Over all, our data demonstrated the possibility of interactions between the herbal extracts and the conventional antidepressants utilized in the present study.

Recently, interactions of herbal medicines with synthetic drugs came into focus of particular interest. Contrary to popular belief that nature is always safe, herbal medicines may cause significant toxic effects and even death as these are often combinations of botanical extracts that are assumed to have additive or synergistic effects (Fugh-Berman, 2000).

The issue is getting a worthy attention all over the world as the herbs have always been the principal form of medicine in developing countries, becoming popular once again on the global scenario and receiving a great deal of attention as alternatives to synthetic pharmaceutical products in recent times leading to increase in their demand (Mythilypriya et al., 2007; Sparreboom et al., 2004; Poppenga, 2002).

The documented evidence demonstrated that herbs can interact with prescribed medications and can put patients at risk (Brazier and Levine, 2003; Izzo and Ernst 2001; Fugh-Berman, 2001). The use of complementary and alternative medicine in treating neurologic disorders has increased in popularity in response to advances in human alternative and integrative therapies and implied the obvious use of alternative techniques coupled with conventional medicine (Kline, 2002). Simultaneously, diverse studies have indicated that interactions between herbal medicines and synthetic drugs exist and can have serious consequences (Patel and Gohil, 2008; Gohil and Patel, 2007; Izzo et al., 2002). Historically, herbs and drugs have been presumed to be very different treatment modalities that have rarely, if ever, been used together (Miller, 1998). The line that separates the use of herbs and drugs, however, has blurred in recent decades as
the lay public gains increased accessibility to multiple treatments. It is not uncommon for one patient to seek care from several health professionals for an ailment. As a result, a patient easily might be taking multiple drugs, herbs, supplements and vitamins concurrently. And as these herbal medicines are being used by an increasing number of patients nowadays, who also, typically do not advise their clinicians of concomitant use making consultation even more difficult, leading to known or potential herb-drug interactions inadvertently. The situation confirms to the suspicion that such interactions certainly do exist and needed to be screened for (Engler et al., 2009).

The literature revealed many such interactions (Izzo, 2004; Williamson, 2003; Izzo and Ernst, 2001; Izzo et al., 2002; Ernst 2000a; Ernst, 2000b). For instance, echinacea could cause hepatotoxicity, if used beyond 8 weeks and therefore it was not advised to be used with other known hepatotoxic drugs, such as anabolic steroids, amiodarone, methotrexate, and ketoconazole (Miller, 1998). Nonsteroidal anti-inflammatory drugs were postulated to negate the usefulness of feverfew in the treatment of migraine headaches. Feverfew, garlic, ginkgo, ginger, and ginseng were claimed to alter bleeding time and therefore advised not to be used concomitantly with warfarin sodium (Heck et al., 2000). Additionally, ginseng was proposed to cause headache, tremulousness, and manic episodes in patients treated with phenelzine sulphate (Jones and Runikis, 1987; Shader and Greenblatt, 1985). Ginseng was also not advised to be used along with estrogens or corticosteroids because of possible additive effects. Since the mechanism of action of St. John’s wort is still uncertain, its concomitant use with monoamine oxidase inhibitors and selective serotonin reuptake inhibitors was ill advised (Dannawi, 2002; Gordon, 1998; Lantz et al., 1999; Markowitz et al., 2001).

Valerian was advised, not to be used concomitantly with barbiturates and benzodiazepines because of the risk of excessive sedation (Mennini et al., 1993; Hozl
and Godau, 1989). Kyushin, liquorice, plantain, uzara root, hawthorn, and ginseng were assumed to interfere with either digoxin pharmacodynamically or with digoxin monitoring (Lucas, 2006; Izzo, 2004; Izzo et al., 2005; Miller 1998). Evening primrose oil and borage were not advised be used with anticonvulsants because they were suggested to lower the seizure threshold (Holman and Bell, 1983). Shankhapushpi, an Ayurvedic preparation, was shown to decrease phenytoin levels as well as diminish drug efficacy (Dandekar et al., 1992). Kava was shown to have additive effects with central nervous system depressants and the same when used with alprazolam was shown to have resulted in coma (Almeida and Grimsley, 1996; Schelosky et al., 1995). A caution was also advised with regards to its simultaneous use with benzodiazepines, barbiturates, antipsychotics and alcohol for the same reason stated (Dinh et al., 2001; Cupp, 1999). Immunostimulants (eg, echinacea and zinc) were not advised to be given along with immunosuppressants (eg, corticosteroids and cyclosporine) (Lininger, 2000; Miller, 1998). Tannic acids present in some herbs (eg, St John’s wort and saw palmetto) were presumed to inhibit the absorption of iron and kelp as a source of iodine was postulated to interfere with thyroid replacement therapies (Miller, 1998). Numerous herbs (eg, karela and ginseng) were proposed to affect blood glucose levels and therefore they were not advised to be used in patients with diabetes mellitus (Miller 1998; Aslam and Stockley, 1979). A recent research study reported the pharmacodynamic interaction of *Momordica charantia* (MC) with rosiglitazone in rats (Nivitabishekam et al., 2009). Both rosiglitazone (2 and 5 mg/kg) and MC (500 mg/kg) administered orally showed a hypoglycemic effect in oral glucose tolerance test which was more potent with the combination of both compared to that of either of the drugs given alone. MC also augmented the hypoglycemic effect of rosiglitazone in both STZ induced diabetes in adult and neonatal rats in the study mentioned. Another recent study has shown that the plant extracts like *Heliopsis longipes* (HLEE) in combined use
with synthetic drugs diclofenac can interact synergistically at the systemic level and that this association may therefore represented a therapeutic advantage for the clinical treatment of inflammatory pain, allowing lower doses and limiting side effects (Acosta-Madrid II et al., 2009).

Complications may arise from the herb’s direct and pharmacodynamic or pharmacokinetic effects. The pharmacokinetic interactions often involve drug metabolizing enzymes and drug transporter systems. On the other hand, pharmacodynamic interactions may occur when an herbal product produces additive, synergistic, or antagonist activity in relation to the conventional drug with no change in the plasma concentration of either herbal product or drug. A synergistic interaction occurs when two drugs with similar properties show an additive or even exponential increase in clinical impact when given together. In contrast, an antagonistic interaction occurs when two drugs with similar properties are administered simultaneously and show lessened or no clinical effectiveness. These synergistic or antagonistic interactions might occur with concurrent use of any medicinal substances, regardless of whether they are herbs, drugs or both. In addition, herbals with the potential to cause organ toxicity may further increase the risk of toxicity when drugs with similar toxicity are administered concurrently, such as when the hepatotoxic herbal comfrey when administered along with large and prolonged doses of acetaminophen was implied to worsen the hepatotoxicity (Rode, 2002).

On the contrary, the studies exists too, that showed a lack of interaction when some herbs or drugs were taken together. For example, many clinical studies showed the pharmacokinetics of alprazolam were not affected by a number of herbal medicines, including green tea, saw palmetto, garlic, ginkgo and Siberian ginseng (Donovan et al., 2004; Markowitz et al., 2003a; Markowitz et al., 2003b; Markowitz et al., 2003c; Donovan et al., 2003). Recently, St. John’s wort was shown to have no effect on the
Because of diverse and conflicting data available in literature, it becomes difficult to predict whether the combination of all these substances will lead to unwanted side effects and/or interactions. It is imprudent to assume that there will be no interactions. On the other hand, it is just as unwise to abandon treatment simply for fear of possible interactions. The solution to this situation is in the understanding of pharmacokinetic or pharmacodynamic herbal drug interactions. By understanding the same, one can recognize potential interactions and take proper actions to prevent their occurrence.

The present study investigated the possibility of such interactions between three important, widely utilized neuroprotective herb extracts of *Bacopa monniera* (BM), *Centella asiatica* (CA) and *Curcuma longa* (CL) with that of commonly prescribed antidepressants fluoxetine, imipramine and reboxetine respectively with an attempt to determine the probable mechanisms behind the nature of interactions observed in the respective combinations evaluated.

**Selection of plants**

*Bacopa monniera* (BM), *Centella asiatica* (CA) and *Curcuma longa* (CL), the plants selected for this study are an outstandingly important medicinal herbs, widely used in orient and becoming increasing popular in the west. The plants occur naturally in India, and have a long history of use in the Ayurvedic medicine tradition in the treatment of a number of disorders, including neurological conditions, particularly those involving anxiety, depression and rejuvenating intellect and cognition (Husain et al., 2007; Singh and Dhawan, 1997).

The herbs have been investigated in several laboratories in India for their various neuropharmacological effects (Husain et al., 2007; Thiyagarajan and Sharma, 2004; Malhotra and Das, 1959; Aithal and Sirsi, 1961; Prakash and Sirsi, 1962).
BM is herb, currently being marketed in Asian and western countries as a memory enhancing agent. Studies have shown that the crude plant extract of the herb contains many active constituents, including a number of alkaloids and saponins named bacosides A and B which were thought to be responsible for its anxiolytic effects (Shankar and Singh, 2000; Bhattachrya and Ghosal, 1998), antidepressant activity (Sairam et al., 2002), anticonvulsive action (Shanmugasundaram et al., 1991) and antioxidant activity (Singh et al., 2006; Bafna and Balaraman, 2005; Rohini et al., 2004). But so far, there are very few studies reporting the role of BM in the functional regulation of neurotransmitters and their receptors or in herb-herb interactions or herb-drug interactions, despite a major caution raised for the same (Izzo, 2004).

The second herb chosen for the study, *Centella asiatica* (CA) is also a traditional Indian medicinal plant, which has been described to possess central nervous system activities, such as intellect and cognition enhancing, anti-oxidant, sedative antianxiety, antidepressant and antiepileptic properties (Ganachari et al., 2004). The primary constituents responsible for the pharmacological effects were thought to be the saponin containing triterpenoids including asiaticoside, a major and the most important component, in terms of quality control of the herb (Zheng and Qin, 2007).

A great progress has been made over the past decades in study of biologically active components, bioactives and mechanisms of CA, but the results are still unsatisfactory. Despite multifarious claims regarding underlying mechanisms involved in biological actions of this herb, still more scientific data are needed to justify its ever increasing use. The therapeutic potential of this plant in terms of its efficacy and versatility is such that further detailed research would appear momentous.

The third herb selected for the present study is *Curcuma longa* (CL), a plant native to India and is the source of the culinary spice known as turmeric, a traditional herbal remedy containing curcumin as an active ingredient (Hatcher et al., 2008).
The aqueous CLE and curcumin were shown to possess many therapeutic properties including antioxidant, anti-inflammatory, immunodulatory, and neuroprotective activities (Thiyagarajan and Sharma, 2004; Xu et al., 2005a; Xu et al., 2005b; Motterlini et al., 2000).

The extracts of the three plants mentioned above were commercially acquired to evaluate the possibility of the interactions with commonly prescribed synthetic antidepressants in the present study, as the same are usually brought off the counter and used therapeutically in the extract form mostly; rather than the purified phytoconstituents.

**Identification of phytoconstituents**

Standardization of herbal medicines and plant ingredients especially with reference to their active marker is the need of time. To minimize batch variations and to assess scientific validity to herbal extracts, it is necessary that herbal drugs should also be analysed like modern drugs and the proper quality control techniques should be applied to verify the quality and quantity of the same, specially, if it is a polyherbal drug. High performance liquid chromatography (HPTLC) is fast emerging as one of the major tool by which the quality control of herb extracts and phytoconstituents in the formulations can be maintained and identification of various chemical markers of the herbs can easily be done (Sane et al., 1998).

Various methods like spectrophotometry (Singh and Dhawan, 1997; Pal and Sarin, 1992), spectrofluorimetry and HPLC (Ganzera et al., 2004; Renukappa et al., 1999; Pal et al., 1998) have been reported for the estimation of bacosides. On the other hand, few methods like gravimetric (Upadhyay et al., 1991) and column chromatography (Singh and Rastogi, 1969) have been suggested for the estimation of asiaticoside, which are not very precise or sensitive methods and require multiple step extractions and
purification, except, that in case of HPLC. A few HPLC methods have also been reported for the quantification of curcumin (Jayaprakasha et al., 2002).

In present study, the presence of active biological constituents in the extracts were identified by TLC followed by HPTLC methods which were validated and reported to be simple rapid, sensitive, accurate, reproducible and cost effective methods for the routine analysis of bacoside (Prakash et al., 2008; Deepak et al., 2005; Shrikumar et al., 2004; Gupta et al., 1998), asiaticoside (Singh et al., 2005) and curcumin (Pathania et al., 2006). Using the proposed methods, several solvent systems were tried to separate active constituents, namely bacosides, asiaticoside and curcumin in BME, CAE and CLE respectively and the ones which provided a good separation and resolutions were used for the extractions. The respective chromatograms were obtained by HPTLC method and three respective peaks obtained for bacosides, asiaticoside and curcumin confirmed the role of aforementioned active constituents in the herbal extracts utilized in the present study.

Selection of drugs

The synthetic drugs chosen to study the interactions with above mentioned herbs are the widely prescribed and the most commonly utilised conventional antidepressants available in the market today, namely fluoxetine, imipramine and reboxetine. They made for an easy choice because, the drugs of most concern for interactions with the herbs were mostly those that people take continuously, especially, for the chronic illness such as depression, a common and disabling disorder which is ranked fourth in a list of the most urgent health problems worldwide, having major effects on economic productivity, individual well being and social functioning around the globe, turning out to be a huge burden on individuals, families, and society (Rao and Chen, 2008; Norman and Burrows, 2007; Kadhe et al., 2003).
The traditional medical treatment for depression are the prescription antidepressant drugs that work by increasing neurotransmission for one or more of the monoamines—serotonin, NA or DA. Before the year, 1980, antidepressant treatment consisted primarily of the tricyclic antidepressants (TCADs), monoamine oxidase inhibitors (MAOI), and lithium (Kadhe et al., 2003). Most of today’s medications are based on the tricyclic antidepressants, which are believed to act by inhibiting the plasma membrane transporters for serotonin and/or NA (Manji et al., 2001; Morilak and Frazer, 2004); but they also have many side effects due to binding with the multiple unrelated receptors. The selective serotonin reuptake inhibitors (SSRIs), an important class of antidepressant introduced in the late 1980's, includes fluvoxamine, fluoxetine, sertraline, paroxetine, and citalopram. The drugs have become a mainstay of antidepressant treatment because of substantial advantages over the tricyclics and monoamine oxidase inhibitors in safety, tolerability, and ease of dosing. These older medications provided a template for the development of newer classes of antidepressant. NA reuptake inhibitors (NRIs) and serotonin and NA reuptake inhibitors (SNRIs) are still used today with great success (Berton and Nestler, 2006). The drugs used in the present studies were, fluoxetine, A SSRI, one of the most effective and widely prescribed medications today (Cryan et al., 2002), a tricyclic antidepressant imipramine, a presynaptic uptake inhibitor of NA as well as serotonin (Baldessarini, 2001) and reboxetine, (ariloxylbenzyl derivative of morpholine), a selective NA reuptake inhibitor (SNaRI), which is the first commercially available drug developed specifically as a first line therapy for major depressive disorder (Hajos et al., 2004).

Choice of animals

Healthy adult, male rats and mice were used in the present investigation. Although, there does not appear to be any significant degree of sex variations in induction of
depression models (Willner, 1991), the male species were used to ensure that the animals belong to a specific species to avoid the variability in results.

**Preclinical toxicity tests**

Even though the acute toxicity test has been widely criticized as a parameter for assaying toxicity (Timbrel, 2002; Klaassen, 2001; Lorke, 1983) there are still certain occurrences when some useful information could be obtained from such studies. Apart from giving a clue on the range of doses that could be used in subsequent toxicity tests, it could equally reveal the possible clinical signs elicited by the substance under investigation. It is also a useful parameter for estimating the therapeutic index (i.e. $LD_{50}/ED_{50}$) of drugs and xenobiotics (Rang et al., 2001).

The present study has shown that the BME, CAE and CLE possessed fairly high oral $LD_{50}$ values, greater than 2g/kg in mice and rats. And in relation to the therapeutic doses mentioned in the folklore, the routine dose of the extracts mentioned were up to 5g/kg daily (Monograph, 2004; Brinkhaus et al., 2000; Martis and Rao, 1992; Kartnig, 1988; Ravindranath and Chandrashekara, 1982).

Thus, their high oral therapeutic index might be used as a rough indicator of wide margin between the effective and toxic dose. However, it was not possible to calculate $LD_{50}$, since the administered volume could not surpass 1 ml in mice under study.

In general, the acute treatment with the herbal extracts used in the study did not show any toxicity as compared to the control group from the beginning until 14th day after the treatment. Such observations indicated the absence of any sequential toxicity of the extracts used in the study. Even though, it was not possible to determine the $LD_{50}$ of the standard extracts, our results revealed low order toxicity of the same.

But such acute toxicity data are of limited value for the clinical application since cumulative toxic effects do occur even at the low dose. Hence, sub acute toxicity studies are always an invaluable tool in evaluating a safety profile of the phytomedicines. This probably explained why some authors have suggested that the subchronic toxicity data is the need of hour to predict the hazards of long term, low
dose exposure to a particular compound (Mcnamara, 1976). The subacute toxicity studies allows the establishment of the existence of any adverse effect and later the same after a prolong exposure. In the subacute toxicity tests performed in our laboraoty, no mortality in animals were observed after the administrations of BME, CAE or CLE up to 2g/kg was probably an indication that this dose level was not toxic to the experimental animals and this with the fact the above dose was about 50 times the therapeutic dose and the very high dose is rarely used ethnomedicinally. This may be an important point in assessing suitability of doses used for the study. The weight profiles of animals also revealed that BME, CAE and CLE did not alter the weights consistently or significantly in animals at the doses used in the study and thus it was decided that the extracts did not have any specific effect on normal body growth of the animals utilized in the study.

**Dosage administrations**

The standard and test doses of herb extracts and drugs were given orally, in graded doses, since this is generally considered to be the likely route of clinical use. A vehicle (distilled water) treated control group and other standard groups, as a group treated with drug alone and the groups treated with the three herb extracts in three different doses, were important for the statistical evaluation and for their comparisons with the groups administered with the combination of these herb extracts along with the drugs.

A single acute drug administration could have been adopted. However, in case of plant extracts, subchronic (1-2 weeks) drug administration was considered obligatory (Bhattacharya et al., 1999b). Moreover, the pharmacotherapy of depression typically requires chronic drug treatment (Mitchell and Redfern, 2005). Multiple acute doses may be administerd over a short period, simply to increase the levels of drug in the biophase without evoking secondary adaptive changes in neurotransmitter mechanisms. In contrast, chronic treatment refers to repeated bolus doses administerd for the extended period of time (days, weeks or months).
The effects of chronic treatment may be associated with drug-induced adaptation in the neurotransmitter-mediated system rather than increased drug levels in plasma. Thus the effect of acute treatment may be just opposite to that seen chronically (due to side effects, like sedation) or absent. Also, irrespective of how it responds to acute treatment; to be valid, a chosen animal model of antidepressant must respond to the chronic treatment. So sub chronic administrations (2 to 3 weeks) of the herbal extracts along with traditional antidepressants were implemented in animal models of depression in the existing study.

**Choice of models and methods**

To study the behaviour is an important tool which is integrated with the biology of the whole animal and like other organs; behaviour ensures the survival and reproductive success of the animal. In so doing, behaviour is intimately involved in homeostasis (Garner, 2005; Baumans, 2005). The reasons for choosing behavioural models like FST, TST and CFT to study the interaction of the herbs with fluoxetine, imipramine and reboxetine and swim stress on NA levels were manifold. First, the plants used in the study were thought to play an important role in behavioural processes related to cognition, mood control, attention and motor performance (Husain et al., 2007). Second, the brain region receives a strong modulatory input from monoaminergic neurotransmitters, and many affective disorders were thought to reflect disruption of the regulation of these processes (Arnsten, 1997; Le Moal and Simon, 1991). Furthermore, from a clinical perspective, it has been postulated that abnormal function in the prefrontal cortex in brain is associated with affective disorders. Imaging studies have revealed differences in this brain region between depressed patients and normal controls (Gillin et al., 2001; Drevets, 2000). The simultaneous monitoring of neurotransmitters in brain alongwith behavioural assessment allows convergent
observations on the neurochemical correlates of antidepressant drug treatment during exposure to stress.

**Biochemical estimations**

Catecholamine (CA) is the name of a group of aromatic amines (NA, Ad, DA and their derivatives) which act as hormones and neurotransmitters. Ad and NA are formed from dopamine which acts on a cardiac musculature and the metabolism (Ad) as well as on the peripheral circulation (NA) and help the body to cope with acute and chronic stress (Kagedal and Goldstein, 1988). An increased production of catecholamines can be found with tumours of the chromattine system (pheochromocytoma, neuroblastoma, ganglioneurona). An increase or decrease in the concentrations of catecholamines can also be found with hypertension, degenerative cardiac disease, schizophrenia and maniac-depressive psychosis (Kagedal and Goldstein, 1988).

CA analysis in tissue, urine cerebrospinal fluid, and blood plasma has been of great importance in understanding of sympathoadrenal function in laboratory animals and in humans. CAs produce many important physiologic effects in the brain, cardiovascular system and other organs. It is therefore essential to be able to elucidate its variation in quantitative chemical tests and in turnover studies. The concentrations of the major neurotransmitters like NA, Ad and DA in biological tissues are modified by physiological factors and influenced by pharmacological agents.

The introduction of high performance liquid chromatography-electrochemical detection (HPLC-ED) has shown to offer high selectivity and sensitivity for the analysis of CAs and related compounds in complex media (Isimer et al., 1991; Kontur et al., 1984; Mefford, 1981). The successful application of immunological techniques was also an important advance for the precise measurement of CAs (Johnson et al., 1980). Besides, fluorimetric and radioenzymatic assays are presently the most widely used techniques for the estimation of plasma, urine, and tissue CAs (Raum, 1984; Cryer, 1984).
The fluorimetric assay lacks specificity and sensitivity. On the other hand, the radioenzymatic assay was reported as significantly more sensitive and specific (Raum, 1984). The availability of sensitive, single-isotope derivative (radioenzymatic) methods for measurement of CAs has stimulated the study of adrenergic mechanisms over the past decade. These methods have been useful for in vivo as well as in vitro studies and the same have complimented new methods for measurements of hormones and metabolic substrate kinetics. Also, it has allowed the controlled studies designed to determine the relative physiological roles of the CAs (Cryer, 1984).

The radio enzymatic immunoassay employed in the present study, utilized an antibody that specifically binds metanephrine. The tissue epinephrine (Ad) and norepinephrine (NA) were detected by the conversion to metanephrine and required only one extraction with alumina to aid in specificity and to concentrate the CAs and sample detection was done by gamma counting. The radioenzymatic assay is presently the reference method for CAs and is best suited for the numbers of samples where sample volume is limited and exquisite sensitivity is required, apart from offering other advantages like savings in cost and time compared to HPLC-ED method. The estimation of serotonin in brain was also described previously, using bioassay procedures (Amin et al., 1954; Twarog and Page, 1953), but previously, it was not possible to measure the fluorescence of compounds, such as serotonin, that are activated in the far ultraviolet region. The compound has never been physically identified in the central nervous system until the spectrofluorimetric method was described for both the identification and assay of this substance in the brain (Bowman et al., 1955).

The development of the spectrofluorimetric method has made it possible to extend fluorescence measurement into portion of the UV region; since, with this instrument, it is possible to deliver high intensity monochromatic light for activation at all wavelengths from approximately 240-800 m\(\mu\) and to carry out automatic spectral
analysis of the resulting fluorescence throughout the same range. Activation of 5-HT occurs maximally at 295 m\(\mu\), and the fluorescence maximum is at 350 m\(\mu\) in dilute acid or at neutral pH (Udenfriend et al., 1955a).

When large amounts of 5-HT are present in the tissue (more than 20 µg per g.), as in animal experiments in which 5-HT or its precursor 5-hydroxytryptophan have been administered or in malignant carcinoid tumours, it is possible to measure the 5-HT colorimetrically utilizing the reaction with 1-nitroso-2 naphthol (Udenfriend et al., 1955a). This is usually performed following the butanol extraction procedure.

The blood levels of 5-HT in man and many other animals are too low to be assayed by the direct protein precipitation method and poor recoveries are obtained if one attempts to extract 5-HT from whole blood into butanol. However, the bulk of the 5-HT in the blood is present in the blood platelets and it is possible to isolate these blood cells and assay them for 5-HT and correlate the amount of 5-HT present to the protein content of these cells (µg. off 5-HT per mg. of platelet protein).

This method was found to be inadequate for the assay of serotonin in brain because other material in this organ was also extracted by butanol and interfered in the fluorescence assay. The finding that serotonin fluoresces at 550 m\(\mu\) in 3N HCl (Udenfriend et al., 1955b) has made it possible to make positive identification of serotonin in mammalian brain and to accurately assay it without interference from other normally occurring material (Bogdansky et al., 1956). However, in 3N HCl, the fluorescence shifts and a new peak appears at 550 m\(\mu\). This shift of fluorescence in strong acid is a function of the phenolic group and distinguishes 5-hydroxyindoles from other closely related compounds and in the solution, it is possible to measure as little as 0.05 µg of 5-HT with fluorescence techniques.

In the present study, the concentrations of NA, Ad in 10% brain tissue homogenates of rodents were measured by the radioimmuno assay and the serotonin was estimated by
the spectrofluorimeter. The results obtained in the present studies indicated the possibility of interactions between *Bacopa monniera* (BM), *Centella asiatica* (CA) and *Curcuma longa* (CL) and the respective conventional antidepressants like fluoxetine, imipramine and reboxetine, when administered simultaneously in lab animals.

To ensure that the activity profile can be referred easily while discussing different aspects of the data generated during the study; the results obtained with different tests have been provided in form of a consolidated statement for the three combinations of herbal extracts and drugs evaluated in animals, as presented in table 1, table 2 and table 3 respectively in this section. (Refer, table 1. A review of the results obtained in first regimen of BME and fluoxetine combinations), (Table 2. A review of the results obtained in second regimen of CAE and imipramine combinations) and (Table 3. A review of the results obtained in third regimen of CLE and reboxetine combinations).
Table 1
A review of the results obtained in first regimen of BME and fluoxetine combinations.

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<thead>
<tr>
<th>Sr. No.</th>
<th>Tests</th>
<th>Group II Drug F-20</th>
<th>Group III BME-20</th>
<th>Group IV BME-40</th>
<th>Group V BME-80</th>
<th>Group VI BME-20+F-20</th>
<th>Group VII BME-40+ F-20</th>
<th>Group VIII BME-80 + F-20</th>
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<td>Regimen BME and fluoxetine</td>
<td>Drug alone</td>
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<td>Combinations</td>
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<td>I: Behavioural assessments</td>
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<td>a. Hyperactivity</td>
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<td>NC</td>
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<td>b. Irritability</td>
<td>↑NS</td>
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<td>c. Straub tail</td>
<td>↑NS</td>
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<td>II. Locomotor activity</td>
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<td>1-week</td>
<td>↑S 0-30 min</td>
<td>NC</td>
<td>↓S 150 min</td>
<td>↓S 150 min</td>
<td>↓NS 150 min</td>
<td>↓S 0 min onwrd</td>
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<td>2-weeks</td>
<td>↓S 180 min</td>
<td>NC</td>
<td>↓S 150 min</td>
<td>↓S 60 onwrd</td>
<td>↓S 120-150</td>
<td>↓S 0 min onwrd</td>
<td>↓S 0 min onwrd</td>
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<td>III. Open field activity</td>
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<td>a. No of Sq crossed</td>
<td>↑NS</td>
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<td>b. No. of rearing</td>
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<td>c. Freezing time</td>
<td>↓S</td>
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<td>↓S</td>
<td>↓S</td>
<td>↓S</td>
<td></td>
</tr>
<tr>
<td>d. Initiation time</td>
<td>↓S</td>
<td>↓S</td>
<td>↓S</td>
<td>↓S</td>
<td>↓S</td>
<td>↓S</td>
<td>↓S</td>
<td></td>
</tr>
<tr>
<td>e. Freq. of Defecation</td>
<td>↓NS</td>
<td>NC</td>
<td>↑NS</td>
<td>↓NS</td>
<td>↓NS</td>
<td>↓NS</td>
<td>↓NS</td>
<td></td>
</tr>
<tr>
<td>4. “Fall off” time</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>15th day</td>
<td>↑NS</td>
<td>↑NS</td>
<td>↑S</td>
<td>↓NS</td>
<td>↑NS</td>
<td>↑NS</td>
<td>↑NS</td>
<td></td>
</tr>
<tr>
<td>5. Forcised swim test (FST)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15th day</td>
<td>↓S 85%</td>
<td>↓S 54%</td>
<td>↓S 64%</td>
<td>↓S 71%</td>
<td>↓S 68%</td>
<td>↓S 77%</td>
<td>↓S 88%</td>
<td></td>
</tr>
<tr>
<td>6. Tail suspension test (TST)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15th day</td>
<td>↓S 65%</td>
<td>↓S 37%</td>
<td>↓S 52%</td>
<td>↓S 55%</td>
<td>↓S 67%</td>
<td>↓S 75%</td>
<td>↓S 82%</td>
<td></td>
</tr>
<tr>
<td>7. Chronic fatigue test (CFT)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>On 5th day of 7 days</td>
<td>↓S 83%</td>
<td>↓S 49%</td>
<td>↓S 36%</td>
<td>↓S 49%</td>
<td>↓S 51%</td>
<td>↓S 92%</td>
<td>↓S 90%</td>
<td></td>
</tr>
<tr>
<td>II. Biochemical estimations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Adrenalin (Ad)</td>
<td>↓S 7%</td>
<td>↓S 7%</td>
<td>↓S 7%</td>
<td>↓S 7%</td>
<td>↓S 5%</td>
<td>↓S 6%</td>
<td>↓S 3%</td>
<td></td>
</tr>
<tr>
<td>9. Noradrenalin (NA)</td>
<td>↑S 59%</td>
<td>↑S 374%</td>
<td>↑S 63%</td>
<td>↑S 789%</td>
<td>↑S 936%</td>
<td>↑S 1472%</td>
<td>↑S 1995%</td>
<td></td>
</tr>
<tr>
<td>10. Serotonin (5-HT)</td>
<td>↑S 17%</td>
<td>↓NS 2%</td>
<td>↑S 25%</td>
<td>↑S 46%</td>
<td>↑S 9%</td>
<td>↑S 28%</td>
<td>↑S 75%</td>
<td></td>
</tr>
</tbody>
</table>

All activities -as compared to the control group
NS = Non significant
S = Significant
NC = Not consistent
↑ = Increase in activity
↓ = Decrease in activity
### Table 2
A review of the results obtained in second regimen of CAE and imipramine combinations.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Drug alone</th>
<th>CAE alone</th>
<th>Combinations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group II</td>
<td>Group III</td>
<td>Group IV</td>
</tr>
<tr>
<td>I. Behavioural assessments</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CAE-100</td>
<td>CAE-200</td>
<td>CAE-300</td>
</tr>
<tr>
<td>1. Gross behaviour</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Hyperactivity</td>
<td>↑NS</td>
<td>↓NS</td>
<td>↓NS</td>
</tr>
<tr>
<td>b. Irritability</td>
<td>NC</td>
<td>↓S</td>
<td>↓NS</td>
</tr>
<tr>
<td>c. Tremors</td>
<td>NC</td>
<td>↑NS</td>
<td>↑NS</td>
</tr>
<tr>
<td>d. Straub tail</td>
<td>↑NS</td>
<td>↑NS</td>
<td>↑NS</td>
</tr>
<tr>
<td>2. Locomotor activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-weeks</td>
<td>↓NS</td>
<td>NC 60 min onwrd</td>
<td>↓S</td>
</tr>
<tr>
<td>3. Open field activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. No of Sq crossed</td>
<td>↑S</td>
<td>↑NS</td>
<td>↑NS</td>
</tr>
<tr>
<td>b. No of rearing</td>
<td>↑NS</td>
<td>↓NS</td>
<td>↓NS</td>
</tr>
<tr>
<td>c. Freezing time</td>
<td>↑NS</td>
<td>↓NS</td>
<td>↓NS</td>
</tr>
<tr>
<td>d. Initiation time</td>
<td>↓NS</td>
<td>↓S</td>
<td>↓NS</td>
</tr>
<tr>
<td>e. Freq. Defecation</td>
<td>↑NS</td>
<td>↑NS</td>
<td>↑NS</td>
</tr>
<tr>
<td>4. “Fall off” time</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15th day</td>
<td>↑NS</td>
<td>↓NS</td>
<td>↑NS</td>
</tr>
<tr>
<td>5. Forced swim test (FST)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7th day</td>
<td>↓S 39%</td>
<td>↑S 62%</td>
<td>↑NS 15%</td>
</tr>
<tr>
<td>15th day</td>
<td>↓NS</td>
<td>↓NS</td>
<td>↓S</td>
</tr>
<tr>
<td>6. Tail suspension test (TST)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15th day</td>
<td>↓NS</td>
<td>↑NS</td>
<td>↓NS</td>
</tr>
<tr>
<td>7. Chronic fatigue test (CFT)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% change On 5th day of 7 days</td>
<td>(\downarrow ) S 1-7 days 80%</td>
<td>(\downarrow ) S #4th day 22%</td>
<td>(\downarrow ) S #5th day 47%</td>
</tr>
<tr>
<td>II. Biochemical estimations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Adrenalin (Ad)</td>
<td>↓NS 7%</td>
<td>↓NS 5%</td>
<td>↓S 7%</td>
</tr>
<tr>
<td>9. Noradrenalin (NA)</td>
<td>↑S 29%</td>
<td>↑S 369%</td>
<td>↑S 548%</td>
</tr>
</tbody>
</table>

All activities -as compared to the control group
NS = Non significant
S = Significant
NC = Not consistent
↑ = Increase in activity
↓ = Decrease in activity
Table 3
A review of the results obtained in third regimen of CLE and reboxetine combinations.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Regimen CLE and reboxetine</th>
<th>Drug alone</th>
<th>CLE alone</th>
<th>Combinations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tests</td>
<td>Group II Drug R-20</td>
<td>Group III CLE-140</td>
<td>Group IV CLE-280</td>
</tr>
<tr>
<td>I. Behavioural assessments</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Gross behaviour</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Hyperactivity</td>
<td>↑NS</td>
<td>↓NS</td>
<td>↓NS</td>
<td>↑NS</td>
</tr>
<tr>
<td>b. Irritability</td>
<td>NC</td>
<td>↓S</td>
<td>↓NS</td>
<td>↓NS</td>
</tr>
<tr>
<td>e. Stereotypy</td>
<td>↓NS</td>
<td>↓NS</td>
<td>↓NS</td>
<td>↑NS</td>
</tr>
<tr>
<td>d. Tremors</td>
<td>↓NS</td>
<td>↓NS</td>
<td>↓NS</td>
<td>↓S</td>
</tr>
<tr>
<td>e. Straub tail</td>
<td>↓NS</td>
<td>↓NS</td>
<td>↓NS</td>
<td>↑NS</td>
</tr>
<tr>
<td>2. Locomotor activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-weeks</td>
<td>↓NS</td>
<td>↓NS</td>
<td>↓S</td>
<td>↑NS</td>
</tr>
<tr>
<td>3. Open field activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. No of Sq crossed</td>
<td>↓NS</td>
<td>↓NS</td>
<td>↓S</td>
<td>↓NS</td>
</tr>
<tr>
<td>b. No of rearing</td>
<td>↓NS</td>
<td>↓NS</td>
<td>↓S</td>
<td>↓NS</td>
</tr>
<tr>
<td>c. Freezing time</td>
<td>↓NS</td>
<td>↓NS</td>
<td>↓S</td>
<td>↓NS</td>
</tr>
<tr>
<td>d. Initiation time</td>
<td>↑NS</td>
<td>↑NS</td>
<td>↑NS</td>
<td>↓NS</td>
</tr>
<tr>
<td>e. Freq. Defecation</td>
<td>↓S</td>
<td>↓NS</td>
<td>↓S</td>
<td>↓NS</td>
</tr>
<tr>
<td>4. “Fall off” time</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15th day</td>
<td>↓NS</td>
<td>↑NS</td>
<td>↓NS</td>
<td>↓NS</td>
</tr>
<tr>
<td>5. Forced swim test (FST)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7th day</td>
<td>↓S</td>
<td>68%</td>
<td>↓NS</td>
<td>18%</td>
</tr>
<tr>
<td>15th day</td>
<td>↓S</td>
<td>↓NS</td>
<td>↑NS</td>
<td>↓S</td>
</tr>
<tr>
<td>6. Tail suspension test (TST)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15th day</td>
<td>↓S</td>
<td>↓NS</td>
<td>↓NS</td>
<td>↓NS</td>
</tr>
<tr>
<td>7. Chronic fatigue test (CFT)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>On 5th day of 7 days</td>
<td>↓S</td>
<td>1-7th day</td>
<td>↓NS</td>
<td>#4th day</td>
</tr>
<tr>
<td>% change on 5th day</td>
<td>61%</td>
<td>46%</td>
<td>40%</td>
<td>61%</td>
</tr>
<tr>
<td>% change on 7th day</td>
<td>44%</td>
<td>37%</td>
<td>21%</td>
<td>61%</td>
</tr>
<tr>
<td>II. Biochemical estimations</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Adrenalin (Ad)</td>
<td>↑NS</td>
<td>↓NS</td>
<td>↑NS</td>
<td>↑NS</td>
</tr>
<tr>
<td>9. Noradrenalin (NA)</td>
<td>↑S</td>
<td>↑S</td>
<td>↑S</td>
<td>↑S</td>
</tr>
</tbody>
</table>

All activities- as compared to the control group.

NS = Non significant.  ↑ = Increase in activity.
S = Significant.  ↓ = Decrease in activity.
NC = Not consistent
Gross behaviour

In gross behaviour test, the groups of rats administered with the combinations of BME in 20, 40 and 80 mg/kg along with fluoxetine 20 mg/kg respectively, showed marked reductions in hyperactivity and irritability in dose dependent fashions. The effects persisted well over 4 hours after drug administrations. The straub tail response was also attenuated in the same groups of rats administered with combinations of BME and fluoxetine. These responses were quite in contrast to the group administered with fluoxetine 20mg/kg alone that showed increase in hyperactivity, irritability and straub tail phenomenon. The groups administered with BME alone in all three doses mentioned above have shown dose dependent reductions too, in hyperactivity, irritability and straub tail but the reductions observed in the groups treated with the combinations of BME and fluoxetine were significantly heightened, specially the last group of rats receiving the highest dose of BME-80 mg/kg along with fluoxetine 20 mg/kg, which showed prominent decrease; compared to that of group receiving BME-20, 40 and 80 mg/kg, alone. The effect may be attributed to the sedative property of the herb due to the saponin fraction bacoside. A previous study has reported a sedative effect of BME attributed to its saponin fraction bacoside A (Malhotra and Das, 1959). Another study also reported the tranquillizing properties of an alcoholic and aqueous extracts of the whole plant in albino rats and dogs (Aithal and Sirsi, 1961). Simultaneously, the drug, fluoxetine also was reported to have sedation as side effect (Erjavec et al., 2000). While both BME and fluoxetine have effects on sedation, wherein the mechanism of action may differ; it may be possible that a combined administration of both in the present study have synergistic neurochemical effects, compounding the effect on the sedation in animals.

On the other hand, the groups of mice administered with combinations of Centella asiatica extracts and imipramine were shown to produce moderate CNS stimulation as
evident from the increase in hyperactivity observed in all animals within 3 hours of the test, after drug administrations, on last day of 2 week regimen. The increase seen was significantly sharper than the groups receiving either CAE 100, 200 and 300 mg/kg alone or than the group receiving only imipramine 20 mg/kg. But at the same time, irritability and the straub tail response were significantly decreased in the same groups as compared to the control group or the groups receiving either CAE or imipramine alone. This was in contrast to the control group, in which animal exhibited a startled response to even very minute stimuli indicating heightened provoked response. The animals in these groups were not provoked by mild or moderate stimuli including slight pinch on skin or frequent poking with a pin.

The groups of mice receiving combinations of CLE and reboxetine also showed similar effects, though the hyperactivity seen were mild and insignificant compared to that of control group or to that of groups receiving only reboxetine or CLE-140, 280 and 560 mg/kg alone, except in groups receiving the highest dose of 560 mg/kg and the groups receiving the same along with reboxetine 20 mg/kg, where the increase was apparent, though, statistically insignificant. The other parameters, like irritability and straub tail incidence in mice receiving these combinations were reduced considerably than control or the groups treated with either reboxetine or CLE extracts alone. Though, the only results which showed statistical significance in this regimen were for the data on decreased irritability observed in group receiving CLE-140 mg/kg, for the reduced tremors seen in groups receiving CLE-280 and 560 mg/kg alone and for the the groups administered with the combinations of CLE-140, 280 and 560 mg/kg along with imipramine-20 mg/kg respectively, where the reduction was more marked compared to that of control group. The stereotypy phenomenon observed in the groups of mice was found to be reduced consistently albeit insignificantly in groups receiving combinations of CLE and imipramine.
The probable mechanisms for mild to moderate hyperactivity could be attributed to the release or stimulation of catecholaminergic receptors at discrete sites in the CNS. It is also possible that the effect may be mediated by the release of excitatory neurotransmitters like glutamate or its congeners like AMPA and NMDA. The other possibility is of involvement of inhibitory neurotransmitter like GABA or stimulation of neurotransmitter like dopamine.

The observations made in this test for all three combinations of herb and respective drugs indicated that all three extracts produced a CNS effects, which were stronger in the groups receiving combinations of the herbal extracts along with the respective drugs than the ones observed in control group or to that of groups administered with the either of the three extracts, namely, BME, CAE and CLE alone, or the groups treated with the conventional antidepressants, namely, fluoxetine, imipramine or reboxetine, alone respectively. The effects of comparatively higher intensity in the groups administered with combinations of herbal extracts with the synthetic antidepressants are suggestive of some interaction at discrete site, at pharmacodynamic level.

**Locomotor activity**

The supplementary symptoms of depression in Diagnostic and Statistical Manual (DSM-IV) that are agreeable to modelling in animals include psychomotor changes, fatigue or loss of energy and disturbances of sleep or food intake (Abramson and Seligman, 1978). Interestingly, it was stated that the psychomotor activity, sleep and appetite may be increased or decreased in depression and the same could be diametrically opposite to the changes in locomotion as cited in support of the validity in different animal models (Mitchell and redfern, 2005). It was suggested that this lack of precision, together with the fact that psychomotor retardation and agitation (which are considerably more complex than gross changes in locomotor activity) may even co-
exist clinically (Nelson, 1981). So it was advocated that a change in locomotor activity as the major, or only behavioural feature, may differ and feature most prominently in many animal models of depression (Willner, 1991). The co-relation between behaviour of mice in porstol’s test and in test of anxiety, locomotion and exploration has also been studied in mice (Hilakavi and Lister, 1990), which suggested that these effects need not be correlated and that the locomotor stimulant may increase swimming behaviour simply by virtue of its motor activating effect rather than a specific effect on behaviour depair and likewise for the locomotor depressants.

The groups of rats treated with BME alone in the doses of 20, 40 and 80 mg/kg alone respectively have also shown the reduction in locomotor activity in dose dependent manner but the reductions were observed to be more marked in the groups administered with the combinations of BME along with conventional antidepressant fluoxetine where these changes were seen very much earlier compared to the groups treated with either BME or fluoxetine alone. The locomotor activity was reduced significantly in the groups treated with the combinations of BME and fluoxetine on 1 week test and the changes were even more profound after 2 weeks, the effect observed, as early as 0 min onwards and persisting till 3 hours of the test.

The reduced locomotor activity could be attributed to sedative and tranquillizing properties of the BME. The combinations of BME along with fluoxetine have shown clear additive effect. The reduced locomotion in groups of rats receiving combinations of BME and fluoxetine in the present study could be attributed to GABA as the BM was said to affect the GABA-ergic system which involves the nerves and synapses of the central nervous system where memory originates and is stored (Shukla et al., 1987). The GABA agonists have already been shown to block the augmented locomotor activity and stereotyped behaviour resulting from dopaminergic stimulation (Agmo et al., 1996; Sandoval and Palermo-Neto, 1995; Cott and Engel, 1977), as GABA was
implicated in the regulation of the activity of dopamine release and inhibition of
dopaminergic activity. Another possibility is that the protein kinase C (PKC) may be
directly involved which is a key regulatory enzyme responsible for modulating both
pre- and postsynaptic neuronal function, synthesis and release of neurotransmitters and
the regulation of receptors (Nishizuka, 1992; Shearman et al., 1989; Rethy et al., 1971).
The possible involvement of GABA and PKC in mediating the action of the herb and
the additive effect observed along with synthetic antidepressant fluoxetine is an
interesting speculation to be explored further.
The groups of mice administered with the combinations of CAE and imipramine, as
well as the groups of mice administered with the combinations of CLE and reboxetine
too showed significant reductions in the locomotor activities from 60 minutes onwards
persistently till 3 hours. The potentiation observed in reducing the locomotor activity
was very apparent in the groups receiving combinations of the above mentioned herbal
extracts along with the respective conventional antidepressants.
A mild CNS depression observed in mice may be due to the presence of multiple active
constituents present in the herbal extracts. The observed activity profile may be due to
multiple mechanisms and site of action of drugs, in addition to the speculations already
mentioned earlier. It would be interesting to probe further in this regard.

**Open field activity**

Tests of emotionality or fearfulness have been widely used in experimental studies of
rodents. The test measures the concept of emotionality, particularly in relation to

In the present study, the groups of rats treated with combinations of BME and
fluoxetine significantly promoted ambulation and the number of rearing, but demoted
freezing time, initiation time and frequency of defecation compared to the groups
treated either with the BME or fluoxetine alone.
The ambulation in open field test was shown to have some relation to fear and anxiety and rearing was attributed to the curiosity in a rodent (Archer, 1973).

From the behavioural observations in the present study, it may be stated that combinations of BME and fluoxetine showed diminished fear, anxiety and curiosity, in the groups of rats. On the other hand, parallel decrease of ambulation and rearing behaviour and freezing time accompanied by increased initiation time were observed in the groups of mice treated with combinations of CAE and imipramine and a distinct decrease of ambulation, and defecation were observed in mice treated with the combinations of CLE and reboxetine. This finding is of particular interest; for, these extracts have the general clinical property of allaying fear and anxiety having multiple mechanisms of actions. There are several reports of investigations concerning exploratory behavior in rats (Rushton and Steinberg, 1966; Rushton and Steinberg, 1963) and familiarity of a rat with an environment. The novel situation of the open field evoked in the animal a pattern of behavior characterized by exploration (ambulation and rearing), emotional defecation and urination (Bindra and Thompson, 1953). It has been considered that the exploration evoked under an unfamiliar environment is modified with psychological factors such as curiosity, fear and anxiety as well as with psychotropic drugs (Rushton and Steinberg, 1966).

In many open field studies, it was commonly assumed that emotionality or fears were inversely related and that the high emotionality inhibits exploration and low emotionality facilitates it. In one study, it was stated that low and high fear states were associated with low exploration whereas the intermediate fear states were linked with very high exploration (Lester, 1968). In another study, it was suggested that high fear facilitates exploration in an elevated maze (Halliday, 1967). On the other hand, a different study mentioned both the possibilities that fear energized responses competing with exploration (for example, freezing) and that it energized ambulation itself.
(Broadhurst and Eysenck, 1964). The probable explanation for the effects observed in groups of animals treated with the respective combinations of herbal extracts and drugs could be the low fear state or reduced anxiety exhibited by the actions of herbal extracts the effects which were noticeably augmented with the simultaneous administrations of the respective conventional antidepressants like fluoxetine, imipramine or reboxetine in the animals.

Moreover, A review of many findings in one study suggested an inverse relationship between defecation and ambulation (Archer, 1973), although the same was not always advocated (Nagy and holm, 1970). Originally, emotionality was related to sympathetic nervous activity (Hall, 1934) but then it was established by many studies that the parameters like ambulation, rearing, freezing and rate of defecation may be either independent or negatively correlated with one another (Archer, 1973). Despite the fact that, the results found in this test were inconsistent and non significant to the major extent, the groups of animals treated with the combinations of herbal extracts and the respective drugs mentioned above, showed the drastic reductions in the frequency of defecation than that of the groups treated with either herb, drug or vehicle alone, suggesting a synergistic effect that resulted from simultaneous administrations of the both.

**Motor coordination**

The increased muscle tone is a common feature of anxiety states in humans. Thus the groups were tested for the effect on muscle coordination and balance in the rota-rod test, on 7th and 15th day after administrations of herbal extracts and drug combinations. The groups of rats receiving BME along with drug fluoxetine showed improved muscle tone compared to the control and rest of the groups compared to that of groups treated with either fluoxetine or BME alone. The effect observed on motor coordination was evidently independent of the locomotor activity which was seen to be reduced in the
groups of rats receiving the same combinations. It is possible that the increased muscle coordination though a specific response to antianxiety or antidepressant activities does not correlate with the reduced locomotor activity observed in the animals. But the response was notably enhanced in the groups treated with the combinations of BME and fluoxetine, nonetheless. On the other hand, the same was reduced significantly in the groups of mice receiving combinations of CAE and imipramine (particularly with the highest dose of CAE- 300 mg/kg) and in groups treated with combinations of CLE and reboxetine, indicating the potentiation of the muscle relaxation. The observed results can be taken as a further evidence for the presence of anti-anxiety activity which was found to be augmented by the groups treated with combinations of respective herbal extracts and the drugs.

Behaviour despair tests

It has been shown previously that behavioural studies play an important role in the evaluation of antidepressant drugs. The FST and the TST are the non-escapable stressful situations which are widely used to predict the clinical efficacy of many types of antidepressant treatments (Karolewicz and Paul, 2001; Porsolt et al., 1978a; 1978b; 1987).

The antidepressant effects of the three herbal extracts in combinations with drugs were checked in behaviour despair test, one of the classical tests for antidepressant described earlier (Porsolt et al., 1978a). The FST was a significant selection because it evokes stress-induced behavioural depression that is sensitive to modification by antidepressant drug treatments.

In all the three combinations evaluated, that of BME and fluoxetine, CAE and imipramine and CLE and reboxetine, the duration of immobility were significantly reduced in animal models of depression.
In group treated with the drug, fluoxetine alone and in groups (III-V) treated with three
doses of BME alone, significant shortening in durations of immobility occurred in rats
in both FST as well as in TST. Similarly, in last three groups (VI-VIII) receiving
combinations of BME and fluoxetine, potentiation in duration of immobility,
especially, at higher dose combination was observed in rats. From this activity profile it
can be unequivocally inferred that the combination of BME and fluoxetine have the
additive effect in reducing the time of immobility.

In groups of mice administered with the combinations of CAE and imipramine also, a
significant reduction in time of immobility was seen, particularly, with the
combinations of imipramine along with the highest dose of CAE- 300 mg/kg on 7th day,
in which the reduction, though apparent, was not found to be significant in another
behaviour test, TST. On the other hand, the effects on the same groups on time of
immobility on 15th day was milder than the control group treated with distilled water, or
the groups treated with respective drugs or herbal extracts alone, which also showed
reductions in time of immobility in dose dependent fashion.

Likewise, in groups of mice receiving an aqueous CLE and its combinations with
synthetic SNRI reboxetine on the immobility behaviours, all the extracts at oral doses
from 140 to 560 mg/kg for 1, 7 and 14 days significantly decreased the duration of
immobility in the FST, modified chronic fatigue test as well as TST. These behavioural
effects in groups treated with the combinations of CLE along with reboxetine were
more potent than that of reboxetine or CLE alone, after 2-week of drug treatments. The
combinations were consistently effective in reducing time of immobility when
evaluated in two classical models of depression in mice, the TST and FST. The
antidepressant effect was clearly potentiated in animals by the aforesaid combinations
indicating synergism at pharmacodynamic level.
It was reported previously that, although all antidepressant compounds reduce behavioural immobility in FST, specific active behaviours elicited in rats by antidepressant compounds could be attributed to pharmacological drug classes; for example, SSRIs increase swimming, and NA-enhancing drugs increase climbing behaviour in rats (Page et al., 1999; Lucki, 1997). Previous studies have also demonstrated complex interactions between the neurochemical effects of forced swimming and the behavioural responses to antidepressant drug treatments and it was reported that fluoxetine treatment altered adaptation of the serotonin response in the lateral septum; changes in extracellular serotonin output were positively correlated with immobility and negatively correlated with swimming but not climbing (Kirby and Lucki, 1997) and that, increased extracellular NA elicited by the FST was negatively correlated with immobility and positively correlated with climbing but not swimming behaviour (Arunrut et al., 2009). These observations supported the mediation of these active behavioural responses to antidepressant drugs in the FST by distinct neurotransmitter systems. However, it has been called to question also, in recent years whether decreased swimming time during the FST reflects behavioral despair, or may denote something altogether different (Holmes, 2003; Cryan et al., 2002), such as a more relaxed mental state, or greater physical fitness for maintaining the floating posture. For example, some research groups have followed decreased swimming time in the FST as an indicator of a lessened stress response (Kelliher et al., 2000).

In the present study, though a specific active behaviour like swimming or climbing could not counted manually in animals, this active behaviours were noted in the animals while evaluating the duration of immobility.

While the groups of rats treated with combinations of BME and fluoxetine showed intense swimming behaviour, the groups of mice receiving the combinations of CAE and imipramine as well as CLE and reboxetine depicted increased climbing behaviour compared to that of groups treated with respective herbs, drugs or vehicle alone.
The subsequent increase in serotonin and NA were also confirmed in animal brains by biochemical estimations performed in the present study.

**Chronic fatigue test (CFT)**

Chronic fatigue syndrome (CFS) is a heterogeneous disorder of unknown etiology characterized by fatigue, neuropsychiatry symptoms and related somatic complaints (Kaur and Kulkarni, 2000). Modified behavioral despair test has been employed to assess the efficacy of the test drugs in this disease condition. The data generated during the study clearly showed that combinations of BME and fluoxetine produced significant synergistic effect in CFT in rats, after 3 weeks.

In CFS, the chronic, concurrent administrations of BME and fluoxetine in combinations in rats, decreased the duration of immobility significantly and more potently than that of other groups receiving either of the drug or BME alone. In this test from day-1 to day-7, of CFT, following 2 weeks of test drug administrations, groups treated with the aforementioned combinations produced superior effects in attenuation of time of immobility in rats in comparison to the control group and the rest of the groups. This clearly indicated the additive effects of BME to the antidepressant effects of fluoxetine in the treatment of chronic fatigue syndrome in rats.

In second regimen of combinations of CAE and imipramine in groups of mice, the data indicated that the herb seems to possess complex activity profile with unpredictable anti-depressant activity potential. However if we consider the results in evaluation of CFT, a slightly different picture emerges; where the chronic administrations of both, the herb CAE along with the conventional drug imipramine showed noticeable reduction in immobility time in mice. In this test from day-1 to day-7, not only the CAE and the imipramine produced a good effect i.e. attenuation of immobility in mice, but also the combination of both CAE and imipramine proved to be very efficacious in decreasing the duration of immobility in both of the tests, namely FST and TST.
In CFT, the significant reductions observed in immobility time in groups of animals receiving combinations of CAE or imipramine compared to that of groups treated with either of them alone, from the first day onwards indicated the important beneficial effect in the experimental chronic fatigue syndrome (CFS).

In the third regimen, the simultaneous administrations of the the combinations of CLE and reboxetine too, showed a great degree of reductions in time of immobility in mice compared to that of other groups receiving either CLE or reboxetine alone, at all three dose levels a indicative of the summation of effects.

CFS has multifactor etiology, important among them are abnormality of hypothalamic – pituitary – adrenal axis (HPA); mild hypocortisolism of central origin, decrease in the plasma level of catecholamine metabolites and increase in the basal level of plasma 5-HT metabolites. It can be suggested that the test drugs in combinations, by acting at several sites in the CFS modulated the factors that were responsible for the appearance of CFS. Since depression too is a heterogeneous disorder with the complex aetiopathogenesis where the exact pathogenesis of depression is yet to be understood clearly, evidences gathered over the years pointed towards the involvement of two monoamine transmitters serotonin and NA. Following the recognition of depressive illness as a biochemical phenomenon, the monoamine hypothesis of depression had become widely favoured (Blier and Abbott, 2001). Several neurotransmitters have been implicated in the pathophysiology of major depression, including the serotonin (5-HT) and NA systems.

Though the neurobiological causes of the disease still remain unclear, several lines of evidences suggested that an enhancement of 5-HT and noradrenergic neurotransmission might be responsible for the therapeutic response to different types of antidepressant treatments (Blier and Ward, 2003; Blier and Abbott, 2001; Blier and de Montigny, 1998).
The noradrenergic mechanisms are thought to be involved in the control of levels of arousal and consciousness, aggression, anxiety and in reward mechanisms too (Montgomery, 1999; Montgomery, 1955).

In the present scenario, the drug types majorly used in the treatment of depression are a) tricyclic antidepressant, b) selective serotonin uptake inhibitor, c) MAO inhibitors and drugs used in bipolar depression like lithium (Mycek et al., 2000). These antidepressant medications are thought to elicit their therapeutic effects by increasing synaptic concentrations of the monoamines serotonin (5-HT) and/or NA (Frazer, 1997). Tricyclic anti-depressants like imipramine inhibit neuronal uptake of monoamines by blocking the uptake 1 which results in increased level of monoamine in the brain leading to alleviation of depression. The fact, that though the above mentioned pharmacological action occurs immediately, it is not reflected in the clinical conditions, for it requires treatment with the drugs for weeks before the anti-depressant activity becomes evident. Because of this reason it has been reasoned that the main mechanism of antidepressant may be the change in the density of monoamine receptors in the brain.

The drugs used in the present study were fluoxetine-a SSRI, imipramine- a tricyclic antidepressant as presynaptic uptake inhibitor of NA as well as serotonin (Baldessarini, 2001) and reboxetine which is a selective inhibitor of NA reuptake (SNRI). It inhibits NA reuptake invitro to a similar extent to the tricyclic antidepressant desmethyylimipramine. Reboxetine does not affect dopamine or serotonin reuptake (Kent, 2000).

The drug was reported to have low in vivo and in vitro affinity for adrenergic, cholinergic, histaminergic, dopaminergic and serotonergic receptors (Holm and Spencer, 1999). To ascertain a possible modulatory effect on the above parameters, effects of herbal extracts, test drugs and the same in the combinations on CFS induced stress related biochemical changes in animals were evaluated in the present study.
In groups of rats receiving BME and fluoxetine combinations, whilst the concentrations of Ad were significantly decreased, the NA and serotonin concentrations were significantly increased in dose dependent fashion in all the groups treated with the parallel administrations of abovementioned combinations. In groups of mice treated with CAE and imipramine combinations, Ad concentrations were decreased albeit nonsignificantly in brain. On the other hand, NA contents were significantly increased dose dependently in brain homogenates of all the three groups of mice treated with the combinations of CAE and imipramine. While, in groups of mice administered with CLE and reboxetine combinations, Ad concentrations were found to be significantly increased, apart from a marked increase in NA in brain.

In one of the previous studies, the biochemical analysis has revealed that chronic swim test significantly increased lipid peroxidation level in whole brains of rats and plus, a decrease in level of total proteins, SOD and glutathione, apart from significantly increasing the level of NA in the brain of experimental animals administered with the combinations of BME and fluoxetine. (Singh and Kulkarni, 2002). Since both catecholamine and 5HT has been implicated in the etiology of depression, the potent additive effects of this herbal extracts and the conventional drug in combinations, in all behaviour despair tests seems to be due to the increased availability of these neurotransmitters at the postsynaptic receptor sites following their re-uptake inhibition. As the results have shown both CNS stimulation and antidepressant effect, it seems that they act in a complex manner modulating several factors. A previous study proposed that the 5-HT receptor activity modulating groups have novel functioning as both anti-anxiety and antidepressants. Anxiety which is an unpleasant state of tension, apprehension or uneasiness, it is among the most commonly encountered mental disturbances. Earlier hypnotic and sedatives were used extensively for the treatment of anxiety. Now, benzodiazepines are the widely used drugs which produce their
pharmacological effects by binding to the site specific for them, situated adjacent to the receptor of GABA (Mycek et al., 2000). Several partial agonists of 5HT1A receptors have been explored for potential utility both in anxiety disorders and in milder cases of mixed anxiety depression (Murphy, 1990). The 5-HT2c serotonin receptors are prominent in limbic forebrain and cerebral cortex. This receptor subtype has been postulated to be a reasonable therapeutic target for depression or anxiety (Murphy, 1990). Among the other mechanisms involved in anxiolytic activity expression is the stimulation of 5-HT1A receptor. This is an inhibitory autoreceptor that reduces the release of 5-HT and other mediators and causes anxiety suppression. Many other neurotransmitters and receptors have been implicated in anxiety and panic disorders, particularly NA and neuropeptide such as CCK and substance P (Sandford et al., 2000). The simultaneous administrations of the combinations of BME and fluoxetine in groups of rats in the present study have shown elevated levels of serotonin as well as NA in brains. The possibility of herbal extracts and test drugs (SSRI) alone and in combinations, acting on 5-HT receptors can not be ruled out. The confirmation of this possibility requires further studies. Possibly, both neurotransmitters 5-HT and NA are involved in antidepressant effects, especially BME, showing potent synergistic effects when used in the combination with the conventional antidepressant fluoxetine. Moreover, selective inhibitors of 5-HT are devoid of the side effects like anticholinergic and cardiotoxic effects often seen with tricyclic anti-depressants. In this particular respect, inhibition of 5-HT up take at 5-HTA receptor subtype has been reported to be critical for the expression of anti-depressant activity. Conceptually speaking drugs stimulating 5-HTA receptor and drugs inhibiting x2-adrenergic receptors by elevating the levels of CA can be therapeutically beneficial in the treatment of depression. A regimen of BME plus fluoxetine combinations was found to be potent in this regard in groups of rats.
The groups of animals administered with the combinations of BME and fluoxetine also showed a significant reduction in Ad concentration which may be responsible for its antistress effect that required further probing. The combinations may help further in dose reduction of the fluoxetine lessening the risk related to SSRIs concerning risk linked with overdosage, though a sedation effect was also found to be prominent in the groups receiving combinations of the herbal extracts BME and the fluoxetine than the groups treated with either the BME or the conventional drug fluoxetine alone which needed to be noted simultaneously.

In groups of mice treated with CAE and imipramine combinations too, the data in the present biochemical assessment showed a significant increase in the level of NA in the mice brain on simultaneous administration of both. The herb CA was reported for its antidepressant activity. In one study, both imipramine and total triterpenes from CA were shown to reduce the immobility time in animals and ameliorated the imbalance of amino acid levels in brain confirming the antidepressant activity of CA (Chen et al., 2003). The same authors investigated the possible antidepressant effect of total triterpenes of CA by measuring the corticosterone levels too in mice brain (Chen et al., 2005). In the study, increased contents of 5-HT, NA, DA and their metabolites 5-HIAA, 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.

It was suggested that MAO enzyme especially in neurons, act as a protective mechanism that limits any excess accumulation of monoamine neurotransmitters caused by the leakage from the synaptic vesicles. A reversible or irreversible inhibition of MAO leads to accumulation of monoamines in the pre-synaptic neurons and escape into the synaptic cleft producing the pharmacological effects (Martin et al., 1987). Based on theory, one of the the probable mechanism behind the effects observed in animals in present study could also be the inhibition of this MAO enzyme which may
explain the increase turnover of the central NA contents in animal brains, the effect which was further augmented in the groups of animals simultaneously administered with the respective herbal extracts and the conventional antidepressants.

Furthermore, CA has been shown to cause significant improvement in both general ability and behavioural patterns in mentally retarded children (Appa Rao et al., 1973) and also in overcoming negative effects of fatigue and stress. In another study, the memory retention effect depicted in groups of rats fed with aqueous CAE was attributed to its effects on the neurotransmitters DA, 5-HT and NA in biochemical analysis of brain homogenates of these animals (Nalini, 1992).

On the other hand, it was known that GABA and its agonists inhibit the central cholinergic action by affecting the turnover rate of acetylcholine in the rat brain (Scatton and Bartholini, 1982), as the Asian CA was reported to increases the cerebral levels of γ-amino butyric acid (GABA), which explains its traditional use as anxiolytic and anti-convulsant. There was also a suggestion based on the results of battery of pharmacological tests carried out on animals, that the actions of the drug may be mediated through the D₂ receptor and cholinomimetic action (Sakina et al., 1990).

However, the results from the behavioural experiments, along with the biochemical analysis in the present study, showed increase NA concentrations in animal brains, implying the release of NA rather than cholinolytic activity as the major involvement in the central action elicited by the herbal extracts.

Additionally, a modulation of activity of the neuroactive steroids has also been reported to have beneficial effect in the treatment of depression (Urani et al., 2001).

Neurosteroids are the steroid hormones that accumulate in the brain independently of endocrine sources and can be synthesized from sterol precursors in nervous cells. The important representatives are progesterone, allopreganolone, pregnanalone and dehydroepiandrosterone (DHEA).
The anti-depressant effect of these neurosteroids has been reported to be mediated through direct interaction with δ - receptors (Urani et al., 2001). Neurokinin receptors were also reported to be involved in the modulation of much emotional behaviour (Steinberg et al., 2001). Selective non-peptide NK, receptor antagonists were reported to show therapeutic efficacy in the treatment of major depression (Kramer et al., 1998).

According to one review, mesolimbic dopaminergic system, in particular the projections from ventral tegmental area (VTA) to nucleus accumbens (NAcc) are reported to play an important role in facilitating the rewarding properties of the drugs with psychostimulant property (Binder et al., 2001). Now it has been a well known fact that most of the psychomotor stimulants are indirect DA-receptor agonists that cause increased DA transmission by binding to the DA transporter, blocking DA uptake or by increasing DA release from the vesicle. D-Amphetamine- being a well-known psychostimulant possesses most of the above-mentioned properties. It can be surmised that the herbal extracts may also share similar properties.

Furthermore, the peptide neurotransmitter, neurotensin has been reported to be having close interrelationship with dopaminergic system especially in the CNS. At most of the sites, neurotensin appears to block the stimulant activity observed with DA stimulation. Administration of neurotensin antagonists were shown to cause potentiation of low methamphetamine induced stimulation of locomotion in a previous study (Binder et al., 2001). While it would be interesting to ascertain whether the herbal extracts contain active principles that would inhibit neurotensin receptors in the CNS to produce the CNS stimulation, the observed activity profile in the present study, could be due to the multiple mechanisms involved as often seen in case of the herbal extracts and also due to the several different sites of action of drugs. It would be interesting to probe further in this regard.
The biochemical analysis in brain homogenates of the groups of mice receiving combinations of CLE along with reboxetine in the present study also showed a remarkable increase in NA concentrations. A preliminary report in previous study stated that curcumin antagonized the syndromes induced by reserpine, such as ptosis and hypothermia that pointed towards the possibility that the antidepressant-like effects of curcumin might be partly due to its influence on the function of adrenergic receptors and/or on the metabolism of NA (Xu et al., 2005a; 2005b; Montgomery, 1999).

The increased concentrations of NA in groups of mice treated with CLE plus reboxetine combinations may be due to additive effects at the receptor level or due to the inhibitory effects on the metabolism of NA leading to its increased turnover in brain. The interesting point to note here in these groups was the significant increase noted in the central Ad concentrations in the groups receiving combinations of CLE and reboxetine. The hyperactivity observed in the animals treated with aforementioned combinations, may be attributed to the increase in Ad, though it required further exploration.

Additionally, CLE was noted to interfere with the way the body processes certain drugs using the liver's "Cytochrome P450" enzyme system, based on previous animal studies (Appiah-Opong et al., 2007) and it was suggested that the levels of these drugs can increase in the blood, and may cause increased effects or potentially serious adverse reactions. This was because the expression of CYP 450 is said to be regulated by both endogenous factors and foreign compounds including drugs and natural compounds such as herbs which are when co-administered consecutively with conventional drugs in modern medicine, can lead to clinically significant herbal drug interactions (Pekthong et al., 2009).
The data generated during the study clearly indicated that the *aqueous* extracts of the herbs BM, CA and CL possess good anti-depressant and anxiolytic activities working on multiple sites of actions and the concurrent administrations of the same along with respective conventional antidepressants, fluoxetine, imipramine and reboxetine clearly potentiated the effects observed in the experimental animals.

Based on all the above account, the augmentations observed in the groups receiving combinations of the three respective herbal extracts along with the synthetic antidepressants in the present study, the probable mechanisms could be postulated as one or more of the following interactions;

a) Inhibition of uptake of monoamines

b) Increased monoamine transmission by binding to the monoamine transporters

c) Increased monoamine release from their vesicles

d) Inhibition of MAO enzyme

e) Stimulation of $\sigma_1$-neurosteroids receptors

f) Blocking of NK, neurokinin receptors

g) Involvement of GABA and DA

As the data suggested, the *aqueous* BME, CAE and CLE in moderate to high doses in combinations with the respective conventional antidepressants can interact synergistically at the systemic level and that this association may therefore represents a therapeutic advantage for the clinical treatment of depression, anxiety or chronic fatigue syndrome. This could also be important in reducing the therapeutic dose of the synthetic drugs to achieve enhanced therapeutic effect with minimal adverse effects, especially, the risk associated with the over dosage of the conventional antidepressants. Some of the side effects like sedation or motor coordination have to be taken in to the account while doing the dose titrations before attempting such combinations.
Consequently, it is tempting to point out that their may be a possibility of such herbal drug interactions, that of herbs utilized in present study, BM, CA and CL along with the newer conventional antidepressant drugs too in the same categories tried in the present study and further research in this area seem prudent.

Even though, there are no documented interactions in literature for the herbal extracts of the plants mentioned above, the speculation of the potential interactions, mainly derived from the laboratory studies can not be negated. Further studies on the herbs, namely, BM, CA and CL, to evaluate the mechanisms of actions on the interaction sites would be quite rewarding in terms of measuring the safety profile and exploring their novel potential therapeutic mechanisms involving probable interactions.

Despite considerable efforts made in the direction of understanding the mechanism of actions for various herbs to justify their diverse use, little attention has been paid to evaluate their potential of interactions with synthetic drugs or even other herbs. More efforts are needed to be focused on this area ignored so far. The process could be complicated than usual, as the most herbs and supplements, unlike conventional drugs are a complex mixture of chemical constituents and most of them have not been thoroughly tested for the interactions with other herbs, supplements, drugs, or foods. Often a complete characterization of the bioactive compounds from an herbal is also unknown.

Additionally, the chemical make up 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 herbal products composed of multiple natural constituents complicate the matter further. Not only does the complex nature of the herbal product complicate the determination of herbal drug interactions, but also the, manufacturing process (e.g., drying process and extraction methods) and batch
variations, that all contribute to the overall complexity (Rosecrans and Dohnal, 2009).

As previously mentioned, because herbal products are not regulated by the 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 (Rosecrans and Dohnal, 2009).

As the herbal medicines are frequently used in combinations with conventional drugs; interactions are likely to be more common than those which manifest clinically. Pharmacokinetic interactions mediated by drug-metabolizing enzymes or transporters are also involved in many herbal drug interactions.

Polymorphisms in the genes for these enzymes and transporters may influence the interactions mediated through these pathways. Herbal drug interactions can be identified in vivo using the herbs with individual probe drugs or drugs with a narrow therapeutic index. A probe drug cocktail approach offers a more efficient screening procedure. If potential interactions are studied in subject groups with different genotypes, more definitive information can be obtained. The selection of genotype groups to study should depend not only on the major enzymes or transporters involved in the disposition of a drug, but also on alternate pathways which may become more important in subjects with reduced activity in the primary pathway. And the pathways for pharmacodynamic effects should definitely be considered for some drugs. Some genotypes may only be found in certain ethnic groups.

Currently, the pharmacogenetic approach has been used mainly to study drug-drug interactions, so there is considerable potential to extend this to the study of herbal drug interactions in vivo studies in humans which are costly, time consuming and may be unethical in certain cases, difficult or may not provide mechanistic information. However, the same are valuable follow up studies to in vitro observations and are the only ones that are most definitive.
**Patient’s guidelines for safety**

While an inadequate reporting makes it difficult to determine whether the herbal drug interaction in question has occurred, even a complete absence of a report in the literature does not guarantee that there is no possibility of such interaction (Fugh-Berman, 2001). However, since such studies are lacking principally, it is hoped that this study in the context of known efficacy studies of selected herbal medicinals along with their potential interactions with the synthetic antidepressants, will serve to alert the physicians to such possibilities of interactions in clinical practice. Clinicians could also include them in their routine drug histories.

Our caution on herbal drug interactions is mainly against the simultaneous use of herbs and drugs that have proven records of interactions. Most of such known interactions are based on reports in scientific publications, laboratory experiments, or on the account of traditional use. It become imperative for the patients to check the package inserts and speak with a healthcare professionals or pharmacists about the possible interactions before using any herbal products along with other medications simultaneously.

It is very important also for pharmacists working in all settings, to be aware of such potentially dangerous herbal drug interactions and other supplement-drug interactions so that any harm to consumers can be minimized.

Some common advices that are important for the patients are as follows (Holcomb, 2009; Eisenberg and David, 1997).

- To avoid regular use of concentrated standardized extracts of herbs, food or nutrients that are known to have the same properties as that of the conventional medications dispensed, and especially the ones that have been reported most frequently to cause interactions in literature.

- Monitoring the appropriate lab tests to check for any unusual toxicity and continue all scheduled blood tests that might help confirm that there would be no problems concerning such interactions.
• While using the herbs and drug together, keeping the maximum intervals about 1 to 3 hours between the administrations of the two, in order to avoid any chance of such interactions.

Also a need of the hour is the standardization and monitoring for adulterations in herbal products to limit the present problem of wide inter-product and intra-product (lot-to-lot) variations in compositions of the active constituents in the same.

At the same time, more scientific studies evaluating efficacy and safety issues on the use of herbal products are needed including the studies directed at herbal drug interactions that would serve public safety. It is of the immense importance that potential herbal drug interactions be identified in order to prevent adverse outcomes in patients taking combinations of drugs and herbal supplements. Such types of preclinical studies; indicative of identification of the mechanisms involved in such interactions will offer future insights in to the approaches to be taken for minimizing their impact and also for designing the appropriate studies in humans.