The present study was designed and carried out to establish the potential neuropharmacological properties of *Amaranthus spinosus*, *Brassica nigra* and *Annona squamosa* extracts as well as ethyl acetate fractions of *Amaranthus spinosus* methanol extract ASP-ME and acetone fraction of *Brassica nigra* ethanol extract BNS-ET and to confirm claims made in traditional system of medicine.

Extraction of dried *Amaranthus spinosus* leaves with different solvent viz. ASL-PE, ASL-CH, ASL-AC and ASL-ME whereas *Brassica nigra* seeds viz. BNS-PE, BNS-CH, BNS-AC and BNS-ET using continuous hot (Soxhlet) extraction method resulted in 1.14, 1.18, 1.20, 1.45, 10.75, 0.80, 0.57, 5.63 % yield of respective extracts while *Annona squamosa* fruit pulp viz. ASP-ME using cold maceration method resulted in 20.00 % yield of extract. Percent yield of ASL-ME (1.45), BNS-ET (10.75) and ASP-ME (20.00) was found better as compared other extracts of relevant plants. Maximum yield of extracts suggested that more number of phytoconstituents is present in particular extract.

The preliminary phytochemical screening revealed the presence of flavonoids, carbohydrates, saponins, triterpenoids, steroids, fixed oil in ASL-PE; carbohydrates, saponins, glycosides, steroids, triterpenoids, fixed oil in ASL-CH; flavonoids, carbohydrates, saponins, glycosides, triterpenoids, steroids, fixed oil in ASL-AC and flavonoids, carbohydrates, saponins, tannins, glycosides, triterpenoids, steroids, fixed oil in ASL-ME, whereas flavonoids, saponins, steroids, triterpenoids, fixed oil in BNS-PE; flavonoids, alkaloids, saponins, steroids, triterpenoids, fixed oil in BNS-CH, BNS-AC and flavonoids, alkaloids, carbohydrates, saponins, tannins, glycosides, steroids, triterpenoids, fixed oil in BNS-ET, while flavonoids, carbohydrates, saponins, tannins, glycosides, triterpenoids, steroids was found to be present in ASP-ME. Earlier reports of chemical constituents and their pharmacology suggest that the plants containing flavonoids, saponins, tannins possess activity against many CNS disorders (Gonzalez-Trujana, et al., 2006). Obtained results of phytochemical profile of above said extracts along with presence of bioactive phytoconstituents for the said disorders supports its ethnopharmacological claims for CNS disorders.
Presence of phytoconstituents was ratified with the help of TLC and HPLC fingerprinting of test extracts and standardized using spectrophotometric methods. TLC fingerprinting profile of ASL-PE, ASL-CH, ASL-AC, ASL-ME, BNS-PE, BNS-CH, BNS-AC, BNS-ET and ASP-ME extracts shown presence of flavonoids, saponins, as well as tannins while HPLC fingerprinting confirmed presence of bioactive phytoconstituents showing same retention time as that of standard caffeic acid (3.875), gallic acid (3.830), quercetin (5.760) and rutin (3.820); ASL-PE shown (3.89, 5.78, 3.82); ASL-CH (3.89, 5.73, 3.82); ASL-AC (3.89, 5.62, 3.82); ASL-ME (3.89, 5.90); BNS-PE (3.827, 3.940, 3.820); BNS-CH (3.820, 3.960, 5.713, 3.820); BNS-AC (3.823, 3.823, 5.700, 3.823); BNS-ET (3.860, 3.860, 6.813 and 3.917) and ASP-ME (3.83, 5.65, 3.83) respectively while as P-hydroxy cinnamic acid were found absent.

In addition, spectroscopic standardization provides information of quantitative analysis of phytoconstituents content. Spectrophotometric quantitative determination of the particular phytoconstituents for standardization was decided on the basis of preliminary phytochemical analysis of respective extract. ASL-PE found to contain 14.40 µg/mg of GAE as total polyphenols and 1.74 µg/mg of RE as a total flavonoids. ASL-CH found to contain 29.24 µg/mg of GAE as total polyphenols and 2.18 µg/mg of RE as total flavonoids. ASL-AC found to contain 16.98 µg/mg of GAE as total polyphenols, and 2.36 µg/mg of RE as total flavonoids. ASL-ME found to contain 4.87 µg/mg of GAE as total polyphenols, and 3.09 µg/mg of RE as total flavonoids. It suggests that ASL-ME contains more bioactive phytoconstituents followed by ASL-PE, ASL-CH and ASL-AC. BNS extracts revealed that, BNS-PE found to contains 52.47 µg/mg of GAE as total polyphenols and 5.33 µg/mg of RE as a total flavonoids. BNS-CH found to contains 47.20 µg/mg of GAE as total polyphenols and 4.61 µg/mg of RE as a total flavonoids. BNS-AC found to contains 38.92 µg/mg of GAE as total polyphenols and 5.45 µg/mg of RE as a total flavonoids. BNS-ET found to contains 68.49 µg/mg of GAE as total polyphenols and 6.60 µg/mg of RE as a total flavonoids. It suggests that BNS-ET contains more bioactive phytoconstituents as compared to BNS-PE, BNS-CH and BNS-AC, while ASP-ME alone found to contains 65.37 µg/mg of GAE as total polyphenols and 5.33 µg/mg of RE as a total flavonoids. Good amount of polyphenols (flavonoids as
well as tannins) have been directly correlated with biological/ pharmacological action.

TLC, HPLC fingerprinting and spectroscopic standardization provides better approach to appraise the quality of the herbal material of concern and caters quality parameter which may be useful in quality control processing such as extraction process of herbal drugs, which has gained intense interest recently (Kroll, 2001; Junhui, et al, 2007).

Test extracts were further screened for antioxidant activity. Antioxidant profile (in vitro) was screened in terms of DPPH scavenging (% DPPH scavenging and % RRI of DPPH) ability. Oxidative stress involves cascade of complex reactions such as excessive production of reactive species (reactive oxygen as well as reactive nitrogen species) in biological systems, which might be due to imbalance between oxidant and/or pro oxidant and defense system, excess production of radicals, degeneration of defense system, diseased conditions and immunological factors. This excessive availability of reactive species in the body thought to lead number of pathological conditions including rheumatic, pulmonary diseases, atherosclerosis, cardiac, CNS disorders and cerebral ischemias. Such excess can trigger chronic inflammatory disorders where potentially reactive species are abnormally produced by activated phagocytic cell (Wojcicki, et al, 1995; Aruoma, 1994; Halliwell, 1994).

Oxidative stress can be counteracted by antioxidant effect produced by mediating one of the following mechanism or in combination thereof viz. by capacity to cause reduction, inhibition of continued hydrogen abstraction, perturbation of chain initiation, decomposition of peroxides and chelation of catalytic transitional metal ions. To understand the means of mechanism of antioxidant behavior single method cannot be helpful, therefore, among the several methods reported, antioxidant effect has been evaluated using different oxidant-antioxidant systems viz., DPPH and percent residual rate of inhibition (% RRI) of DPPH (Shahidi & Wanasundara, 1992; Sanchez, et al, 1999; Soares, et al, 1997).

All the extracts were exhibited reduction of pink colored free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) to the yellow-colored diphenyl picryl hydrazine at varied extents which was measured as absorbances and calculated as percent inhibition. The percent DPPH scavenging activity possessed by standard
antioxidant ascorbic acid, ASL-PE, ASL-CH, ASL-AC, ASL-ME, BNS-PE, BNS-CH, BNS-AC and BNS-ET was found to be 99.12, 52.95, 62.10, 70.40, 73.80, 71.97, 71.91, 81.20 and 92.67 % respectively at the concentration 150 µg/ml. ASL-ME and BNS-ET exhibited better antioxidant activity than the other ASL and BNS extracts while ASP-ME alone exhibited 91.67 % scavenging at 150 µg/ml.

Evaluation of % RRI of DPPH helps to measure the ability of test extracts to scavenge radicals at different time interval such as 50, 90 and 150 min (Yamasaki et al, 1994). ASL-ME showed highest % RRI of 46.24%, followed by ASL-PE (56.20%), ASL-CH (68.35%) and ASL-AC (62.43%) at 50 min. BNS-ET showed highest % RRI of 66.42% as compared to BNS-PE (67.30%), BNS-CH (48.65%) and BNS-AC (73.35%) at 50 min. ASP-ME showed % RRI of 62.24% at 50 min suggesting the ability of ASL-ME, BNS-ET and ASP-ME to scavenge the free radical and protecting biological component from invading action for longer duration as compared to other extracts.

DPPH assay is one of the widely accepted and commonly used models to evaluate antioxidant activity in a relatively short time compared to other methods. This assay involves the formation of stable diamagnetic molecule by accepting an electron or hydrogen radical, which alters color intensity of the reaction mixture. The change in color intensity is proportional to the reduction of pink colored 2, 2-diphenyl-1-picrylhydrazyl (DPPH) to the yellow-colored Diphenyl picryl hydrazyl (Soares, et al, 1997). Measurement of percent RRI is another method which measures the ability of test compounds and extracts to scavenge radicals which may indicates the potential to protect biological system at different time interval (Yamasaki, et al 1994). Results obtained from both the studies suggests that test extracts (specifically ASL-ME, BNS-ET and ASP-ME) are having ability to scavenge the DPPH radicals at different concentrations as well as scavenges and stabilizes the DPPH radicals at different time intervals, which implies role of extracts as antioxidant which may be effective for long duration. DPPH scavenging and % RRI of DPPH of extracts may relate to its marked antioxidant activity, which may provide improved protection to biological system. All these in vitro antioxidant studies imply the high positive correlation between phytoconstituents of ASL, BNS as well as ASP extracts revealing the varied antioxidant ability extracts.
By blocking dopamine receptors provoke an increase in the turnover of dopamine which may lead to the formation of reactive oxygen species (ROS) like hydrogen peroxide, and superoxide radicals (Naidu, et al., 2002). This increased levels of ROS might negatively affect both neurotransmission and cell viability that induce oxidative changes to lipids, resulting in the alteration in membrane functions, protein damage and reduction in intracellular antioxidant defense enzymes. Support to the ‘free radical hypothesis’ comes from in vitro studies where haloperidol induced oxidative stress and vitamin E attenuated neuroleptic-induced changes in striatal monoamine metabolism and protected against neuroleptic induced cell death (Andreasen & Jorgensen, 2000).

A result of in vitro antioxidant suggests that ASL-PE, ASL-CH, ASL-AC, BNS-PE, BNS-CH and BNS-AC posse’s weak anti-oxidant activity as well as also showed presence of fewer amounts of polyphenols as compared to ASL-ME and BNS-ET; which showed correlation between amount of polyphenols and antioxidant activity and hence excluded for further studies. ASL-ME and BNS-ET selected for further studies, which is supported by contents of phytoconstituents determined by spectrophotometric standardization, TLC and HPLC fingerprinting. ASP-ME was the only extract of ASP showed good amount of polyphenols and antioxidant activity; hence ASL-ME, BNS-ET and ASP-ME pooled out and further evaluated for acute toxicity, sub-acute toxicity study, and on the basis of results obtained dose was determined for in vitro and in vivo Pharmacological screening.

Acute toxicity study as per OECD guideline 423 was carried out. The principle of test is to estimate the acute toxicity of a chemical in estimating its median lethal dose. The extracts of ASL-ME, BNS-ET and ASP-ME did not show any toxic symptoms, changes in behavior or mortality in animals even at the maximum dose 2 g/kg p.o. This suggests that the plant extracts are safe even at the highest tolerable doses suggesting a LD50 above 2.0 g/kg by p.o. route. The substances that present LD50 higher than 2.0 g/kg by oral route can be considered practically non-toxic. No toxicity signs or deaths were recorded during the observation period by ASL-ME, BNS-ET and ASP-ME extracts given orally with dose of 2 g/kg.

Sub-acute repeated dose 28-day oral toxicity study was carried out as per OECD Guideline No. 407. Neither absolute body weight loss nor body weight
gain was affected by ASL-ME, BNS-ET and ASP-ME throughout the study. A similar lack of toxic effect was observed in the case of food and water consumption. ASL-ME, BNS-ET and ASP ME at the doses used did not produce any marked changes in rats. There was also no change observed in blood pressure. The administration of ASL-ME, BNS-ET and ASP-ME did not induce any changes in glucose, SGOT, SGPT, cholesterol, triglycerides, HDL, VLDL. No sign of toxicity was observed in case of hematological parameter and organ body weight of control and extract treatment group. All animals survived until the scheduled euthanasia and no gross pathological alteration was found in the internal organs. Organ weight revealed that ASL-ME, BNS-ET and ASP-ME at the doses used did not produce organ swelling, atrophy or hypertrophy. Moreover, the microscopic evaluation did not find any abnormalities in the 2 g/kg ASL-ME, BNS-ET and ASP-ME groups compared to control group.

The acute and subacute oral administration of ASL-ME, BNS-ET and ASP-ME did not induce significant alterations in almost all biochemical, hematological and morphological parameters. Thus the methanol extract of *Amaranthus spinosus* L., ethanol extract of *Brassica nigra* L. and methanol extract of *Annona squamosa* L. was found to be safe in acute and subacute toxicities in experimental animals. Chronic toxicity, mutagenicity and carcinogenicity studies are further necessary to support the safe and sound use of these plants.

In the present study, the effect of ASL-ME, BNS-ET and ASP-ME was studied on various *in vitro* preparations to assess its effect on various receptors and neurotransmitters.

Dopamine and serotonin at (100 µg/ml) log dose of 2.5 exhibited 80.80 and 94.60 of percent response while ASL-ME at (25 mg/ml; 0.5ml) in presence of dopamine and serotonin showed 60.00 and 55.00 of percent response indicating ASL-ME is having ability to significantly inhibit dopamine and serotonin induced contraction on isolated rat vas deferens and rat fundus respectively, whereas Ach produces 87.60 percent response, while ASL-ME in presence of Ach potentiates response and produces 127.20 percent response. The significance was observed mainly at all log dose for dopamine and Ach whereas log dose (1.6 to 2.5) for serotonin.

Dopamine and serotonin at (100 µg/ml) log dose of 2.5 exhibited 90.80 and 88.40 of percent response while BNS-ET at (25 mg/ml; 0.5ml) in presence of
Dopamine and serotonin showed 72.40 and 67.40 of percent response indicating BNS-ET is having ability to significantly inhibit dopamine and serotonin induced contraction on isolated rat vas deferens and rat fundus respectively. The significance was observed mainly at log dose (1.3) for dopamine and log dose (1.9) for serotonin. This outcome helps to contemplate the anti-dopaminergic as well as anti-serotonergic activity mediated through dopamine D$_2$ and 5-HT receptors. Cholinergic M$_3$ receptors are predominantly present in isolated goat tracheal chain preparation. Ach produces dose dependent contractions of isolated goat tracheal chain preparation. The result of the \textit{in vitro} test indicated that BNS-ET potentiated ach-induced contractions on goat tracheal chain preparation. Ach alone produces 89.20 percent response, while BNS-ET in presence of Ach potentiates response and produces 125.80 percent response. The major difference between dopamine, serotonin and Acetylcholine in previous two effects has been inhibited by BNS-ET while the later is potentiating. Experimental results are in accordance to previous theories supporting neuroprotective effect of BNS-ET against dopamine and serotonin, where as potentiating for Acetylcholine.

Dopamine and serotonin at (100 µg/ml) log dose of 2.5 exhibited 104.60 and 134.80 of percent response while ASP-ME at (25 mg/ml; 0.5ml) in presence of dopamine and serotonin showed 78.60 and 86.20 of percent response indicating ASP-ME is having ability to significantly inhibit dopamine and serotonin induced contraction on isolated rat vas deferens and rat fundus respectively, whereas Ach produces 106.90 percent response, while ASP-ME in presence of Ach potentiates response and produces 141.80 percent response. The significance was observed mainly at log dose (1.0, 2.5) for dopamine and log dose (2.2, 2.5) for serotonin as well as Ach.

Dopamine D$_2$ receptors are predominantly present in vas deferens. The vas deference of rat is supplied with hypogastric nerve. The muscle contains dense plexus of catecholamine containing neurons. Dopamine produces dose dependant contractions of vas deferens (Goyal, 2003). The result of the \textit{in vitro} test indicates that ASL-ME, BNS-ET, and ASP-ME inhibited dopamine induced contractions on rat vas deferens. Thus, it is concluded that the ASL-ME, BNS-ET and ASP-ME possess antidopaminergic activity through dopamine D$_2$ receptor blocking.
Rat fundus is a very sensitive tissue for the study of the action of several naturally occurring substances like 5-HT. Rat fundus is slow contraction and slow relaxing type of tissue. Serotonergic 5HT\textsubscript{2} receptors are predominantly present in isolated rat fundus. Serotonin produces dose dependent contractions of isolated rat fundus (Kulkarni, 1999). ASL-ME, BNS-ET and ASP-ME significantly inhibited serotonin-induced contractions on rat fundus. Thus possesses antiserotonergic activity.

The goat tracheal chain is one of the ideal preparations for the study of cholinergic drugs. The tracheal chain is also slowly contracting and relaxing type of tissue. Cholinergic M\textsubscript{3} receptors are predominantly present in isolated goat tracheal chain preparation. Acetylcholine produces dose dependent contractions of isolated goat tracheal chain preparation (Kulkarni, 1999). The result of the in vitro test showed ASL-ME, BNS-ET and ASP-ME potentiated acetylcholine-induced contractions on goat tracheal chain preparation therefore potentiated cholinergic transmission. From the in vitro results, the effect of ASL-ME, BNS-ET and ASP-ME was further studied on various in vivo models to establish its neuropharmacological profile.

In the present work, the effect of ASL-ME, BNS-ET and ASP-ME was studied in several behavioral animal models like elevated plus maze and light/dark paradigm for its anxiolytic property. Anxiolytic effect of ASL-ME, BNS-ET and ASP-ME has been studied using elevated plus maze in terms of time spent (sec) in open arms, enclosed arms and central zone as well as entries in open arms and enclosed arms.

The vehicle treated group rats spent 43.67 sec in open arms, 317.5 sec in enclosed arms and 17.67 sec in central zone while count 9.16 entries to the open arms and 27.67 entries to the enclosed arms respectively.

ASL-ME (100 and 200 mg/kg) and diazepam (1 mg/kg) showed significant ($P < 0.05$, $P < 0.001$) increase in the occupancy in the open arms (130.0, 163.8 and 199.3) as well as in central zone (35.00, 40.83 and 56.67), while decrease in enclosed arm 128.8, 109.5 and 60.17 sec respectively. ASL-ME (50 mg/kg) showed insignificant decrease in time spent in enclosed arms (243.2 sec) and increase in open arms (45.83 sec) as well as central zone (20.33 sec). The animals treated with diazepam (1 mg/kg) and ASL-ME (100, 200 mg/kg) exhibited significant increased count of entries to the open arms (30.33,
19.83 and 24.83) and decreased preference of entries to the enclosed arms (8.83, 12.83 and 10.00) with insignificant change at ASL-ME 50 mg/kg entries in open arms (15.83) and entries in enclose arms (25.50).

BNS-ET (100 and 200 mg/kg) and diazepam (1 mg/kg) showed significant ($P < 0.05$, $P < 0.001$) increase in the occupancy in the open arms (134.2, 184.3 and 199.3) as well as in central zone (36.33, 41.50 and 56.67), while decrease in enclosed arm 131.5, 117.2 and 60.17 sec respectively. BNS-ET (50 mg/kg) showed insignificant decrease in time spent in enclosed arms (245.3 sec) and increase in open arms (55.83 sec) as well as central zone (22.17 sec). The animals treated with diazepam (1 mg/kg) and BNS-ET (100, 200 mg/kg) exhibited significant increased count of entries to the open arms (30.33, 20.50 and 25.51) and decreased preference of entries to the enclosed arms (8.83, 14.83 and 13.00) with insignificant change at BNS-ET 50 mg/kg entries in open arms (16.67) and entries in enclose arms (15.50).

ASP-ME (100 and 200 mg/kg) and diazepam (1 mg/kg) showed significant ($P < 0.05$, $P < 0.001$) increase in the occupancy in the open arms (199.3, 99.17 and 182.8) as well as in central zone (30.17, 40.83 and 56.67), while decrease in enclosed arm 160.0, 108.0 and 60.17 sec respectively. ASP-ME (50 mg/kg) showed insignificant decrease in time spent in enclosed arms (244.8 sec) and increase in open arms (52.33 sec) as well as central zone (19.00 sec). The animals treated with diazepam (1 mg/kg) and ASP-ME (100, 200 mg/kg) exhibited significant increased count of entries to the open arms (30.33, 14.83 and 27.83) and decreased preference of entries to the enclosed arms (8.83, 15.17 and 8.50) with insignificant change at ASP-ME 50 mg/kg entries in open arms (9.66) and entries in enclose arms (22.33).

The etiology of the most anxiety disorders, although not fully understood, has come into sharper focus in the recent past. The benzodiazepines (BZDs) are relatively safe and widely used anxiolytic agent. These agents are known to act through the BZD-GABA receptors. The role of GABA in the anxiety is well established (Rang, et al., 2003). The EPM is one of most popular animal test for research on behavioral pharmacology of anxiety. It involves spontaneous or natural aversive stimuli i.e. height, unprotected opening and novelty (Dhonnchadha, et al., 2003). Several plants are reported to increase the exploration of open arms in the EPM test and are used to diminish anxiety in folk
medicine. The conventional plus maze is highly sensitive to the influence of both anxiolytic and anxiogenic drugs acting at the GABA<sub>A</sub>-BZDs complex. In EPM, rats will normally prefer to spend much of their allotted time in the closed arms. This preference appears to reflect an aversion towards open arms, generated by fear of open space. Drugs that increase open arm exploration are considered as anxiolytics and the reverse holds true for anxiogenics (Hellion-Ibarrola, et al., 2006). In EPM test, the plants extracts ASL-ME, BNS-ET and ASP-ME increased the exploration and the time spent in open arm. The number of entries and time spent in the enclosed arms were reduced by ASL-ME, BNS-ET and ASP-ME (100, 200 mg/kg), in comparison to control values. The anxiolytic effect of the plant extract was more prominent at 200 mg/kg respectively. In this study, diazepam treated animals increased the number of open arm entries, reducing the natural animal’s aversion to the open arms and promoting the exploration thereof, indicating an anxiolytic effect.

Anxiolytic activity of ASL-ME, BNS-ET and ASP-ME has also been evaluated using light dark model.

In present study animals treated with ASL-ME (100 and 200 mg/kg) and diazepam (1 mg/kg) showed significant ($P < 0.05$ and $P < 0.001$) increase in time spent in light zone (39.50, 200.8 and 234.0), number of crossing (35.00, 52.83 and 60.83), transfer latency (31.17, 51.17 and 57.50) respectively as compared to control (17.17, 2.50, 12.17), while significant decrease in time spent in dark zone (169.3, 65.83 and 79.00) was observed as compared to control (217.5).

In present study animals treated with BNS-ET (100 and 200 mg/kg) and diazepam (1 mg/kg) showed significant ($P < 0.05$ and $P < 0.001$) increase in time spent in light zone (41.50, 204 and 234), number of crossing (35.00, 52.83 and 60.83), transfer latency (33.00, 53.33 and 57.50) respectively as compared to control (17.17, 2.50, 12.17), while significant decrease in time spent in dark zone (189.5, 179.7 and 79) was observed as compared to control (217.5).

In present study animals treated with ASP-ME (100 and 200 mg/kg) and diazepam (1 mg/kg) showed significant ($P < 0.05$ and $P < 0.001$) increase in time spent in light zone (63.83, 207.2 and 234), number of crossing (18.17, 47.50 and 60.83), transfer latency (27.33, 51.33 and 57.50) respectively as compared to
control (17.17, 2.50, 12.17), while significant decrease in time spent in dark zone (170.7, 95.67 and 79) was observed as compared to control (217.5).

The dose 50 mg/kg of ASL-ME, BNS-ET and ASP-ME did not produce any significant change in any of parameters.

The light and dark paradigm is based on the natural aversion of rats to bright lit places. Since anxiolytics reduce the natural aversion to light and increase the time spent in the lit compartment. It has been suggested that 5HT₃ receptors may be involved in the fear provoked by L/D paradigm and that the nucleus accumbens or the amygdala may be involved in mediating the disinhibitory effect of 5HT₃ receptor antagonists (Dhonnchadha, et al., 2003). In this model, ASL-ME, BNS-ET and ASP-ME produced significant increase in time spent in lit box as compared to vehicle, showing an anxiolytic activity.

Combining results of all the anxiolytic evaluation assist to contemplate the anxiolytic action of ASL-ME, BNS-ET and ASP-ME is comparable to standard drug diazepam.

The anti-depressant effect of ASL-ME, BNS-ET and ASP-ME extracts was evaluated by using forced swim test (FST) and tail suspension test (TST). Depression is a mood disorder that causes a disturbance in an individual's emotions and feelings. In depressive state there is diminished level of central catecholamine level and also the lower level of 5-hydroxyindolacetic acid which is a principle serotonin metabolite. This suggests that depression is associated with a reduction in central 5-HT function.

The forced Swim test (FST) is a behavioral test widely used to screen new potent antidepressant drugs in rats and mice (Porsolt, et al., 1979). This test is sensitive and specific to all major classes of antidepressant drugs including tricyclic antidepressants, serotonin specific reuptake inhibitors and monoamine oxidase inhibitors (Borsini & Meli, 1988; Detke, et al., 1995). There are extensive evidences to show that different types of experimental stressors induce neurochemical and hormonal changes in animal models of depression (Katz, et al., 1981). The forced swimming test in rats and mice induces alterations in the levels of monoamine and indoleamines, which are reminiscent to human symptoms of depression. There are reports to show that the antidepressant drugs are effective in preventing the behavioral and biochemical changes induced by FST (Miura, et al., 1993; Imperato, et al., 1994). The rats forced to swim in a
restricted space from which they cannot escape and induce a characteristic behavior of immobility. The rats were become immobile, ceased struggling and remained floating motionless in water making only those movements necessary to keep its head above water. This behavior reflects state of depression which reduced by several agents which are therapeutically effective in depression (Hirani, et al., 2002; Porsolt, 1977).

The tail suspension test (TST) is another behavioral test widely used to screen new potent antidepressant drugs in rats and mice. The immobility displayed by rodents when subjected to an unavoidable and inescapable stress has been hypothesized to reflect behavioral despair which in turn may reflect depressive disorder in human. Clinically effective antidepressant reduces the immobility, in TST when mice display after active and unsuccessful attempts to escape, suspended by the tail (Steru, et al., 1985).

Animals treated with ASL-ME (100 mg/kg, 200 mg/kg) and imipramine (15 mg/kg) significantly ($P < 0.05$, $P < 0.001$) decreased duration of immobility in tail suspension test (110.5, 47.00 and 43.17) and in force swim test (65.67, 28.50 and 18.83) respectively as compared to control (143.70 and 128.83). Whereas increased latency of immobility in tail suspension test (99.67, 109.2 and 113.2) and in force swim test (54.83, 121.8 and 57.67) respectively as compared to control (31.00 and 7.83).

Animals treated with BNS-ET (100 mg/kg, 200 mg/kg) and imipramine (15 mg/kg) significantly ($P < 0.05$, $P < 0.001$) decreased duration of immobility in tail suspension test (109.2, 54.83 and 43.17) and in force swim test (96.83, 23.17 and 18.83) respectively as compared to control (143.70 and 128.83). Whereas increased latency of immobility in tail suspension test (90.67, 99.17 and 113.2) and in force swim test (14.17, 36.17 and 57.67) respectively as compared to control (31 and 7.83).

Animals treated with ASP-ME (100 mg/kg, 200 mg/kg) and imipramine (15 mg/kg) significantly ($P < 0.05$, $P < 0.001$) decreased duration of immobility in tail suspension test (105.3, 54.83 and 43.17) and in force swim test (95.83, 38.00 and 18.83) respectively as compared to control (143.70 and 128.83). Whereas increased latency of immobility in tail suspension test (104.0, 114.5 and 113.2) and in force swim test (63.50, 114.2 and 57.67) respectively as compared to control (31 and 7.83).
Administration of ASL-ME, BNS-ET and ASP-ME (100, 200 mg/kg) and imipramine (15 mg/kg) significantly increased latency of immobility and decreased duration of immobility in FST and TST whereas 50 mg/kg showed no statistical significant in duration of immobility as well as latency of immobility.

Combining results of evaluation assist to contemplate the antidepressant action of ASL-ME, BNS-ET and ASP-ME is comparable to standard drug diazepam.

PTZ is the most frequently used substance, and a PTZ-induced convulsion is an acute experimental model in a preliminary screening to test potential anticonvulsant drugs (Gonzalez et al., 2006). The PTZ-induced seizures are similar to the symptoms observed in the absence seizures and the drugs useful in the treatment of absence seizures; suppress PTZ-induced seizures (Rang, et al., 2006).

Anticonvulsant activity of ASL-ME, BNS-ET and ASP-ME has also been evaluated using PTZ induced convulsion model. Study reveals that ASL-ME, BNS-ET and ASP-ME (100 and 200 mg/kg) and diazepam (1 mg/kg) significantly ($P < 0.001$) delayed the onset initially in myoclonic (170.0, 260.4 and 286.6), (116.2, 223.0 and 286.6) and (163.2, 238.6 and 286.6) respectively and in clonic spasms (174.0, 308.0 and 466.2), (137.6, 233.4 and 466.2) and (185.8, 318.0 and 466.2) respectively; when compared with control group treated with PTZ (80 mg/kg) induced myoclonic (50.20) and clonic spasm (48.40).

ASL-ME, BNS-ET and ASP-ME delayed occurrence of seizures and reduced mortality indicating its anticonvulsant potential. Diazepam, a GABA$_A$ agonist is highly effective to prevent convulsions induced by PTZ. The plant extracts may prove useful in treatment of absence seizures and anxiety. The anticonvulsant effects of Amaranthus spinosus, Brassica nigra and Annona squamosa extracts mentioned here may be related to its possible interaction with GABA-Benzodiazepine receptor complex (Ayoka, et al., 2006).

Antipsychotic activity of ASL-ME, BNS-ET and ASP-ME has been evaluated using lithium sulphate induced head twitches and haloperidol induced catalepsy models.

Lithium sulphate administered to rats releases serotonin from serotonergic neurons that stimulates serotonin receptors and produces head twitches. These
head twitches are antagonized by drugs that blocks 5-HT receptors (Kasture, 2006).

In present study the vehicle treated group rats, lithium sulphate produced 49.80 head twitches whereas ASL-ME at 100 and 200 mg/kg shown 17.80, 10.00, BNS-ET at 100 and 200 mg/kg shown 20.00, 9.20 and ASP-ME at 100 and 200 mg/kg shown 17.80, 8.20 respectively; which is significantly \( P < 0.001 \) decreased number of head twitches. Ondansetron (5-HT\textsubscript{3} antagonist) also reduced the number of head twitches (8.00) showing its effect on serotonergic system.

The results showed that at 100 and 200 mg/kg dose ASL-ME, BNS-ET and ASP-ME extracts significantly decreased lithium-induced head twitches. The extract also inhibited contractions induced by serotonin on rat fundus. Thus, suggesting possible inhibitory effect of ASL-ME, BNS-ET and ASP-ME extracts on serotonergic system.

Haloperidol, a typical neuroleptic produces catalepsy in rodents and extrapyramidal side effects in human (Herbert, 2002). Haloperidol-induced catalepsy is one of the animal models to test the extrapyramidal side effects of antipsychotic drugs (Kumar & Kulkarni, 2006). The haloperidol, (a non-selective D\textsubscript{2} dopamine antagonist) induced catalepsy is primarily due to blockade of dopamine receptors in the striatum. The agent, increasing dopamine transmission inhibits neuroleptic induced catalepsy (Rang, et al., 2006). The striatum and nucleus accumbens have been implicated as the major brain structures involved in antipsychotic induced catalepsy, which appears due to the blockade of dopamine neurotransmission (Costall, et al., 1972).

In vehicle treated animals, haloperidol (1 mg/kg) produced maximum catalepsy (224.60) after 90 min. The ASL-ME (50, 100, 200 mg/kg) significantly potentiated haloperidol induced catalepsy at each time interval (0-180 min at 30 min interval) in dose dependent manner. ASL-ME at dose 50 and100 mg/kg showed maximum cataleptic scores 253.00 & 279.00 respectively at 120 min \( P < 0.001 \) in haloperidol treated animals; whereas 200 mg/kg showed 290.80 at 90 min \( P < 0.001 \). However, only ASL-ME at 200 mg/kg did not exhibit any catalepsy and appeared same as the normal animals.

In vehicle treated animals, haloperidol (1 mg/kg) produced maximum catalepsy of 206.00 sec. The BNS-ET (50, 100, 200 mg/kg) significantly potentiated haloperidol induced catalepsy at each time interval (0-180 min at 30
in dose dependent manner 237.40, 270.80 and 306.60 respectively ($P < 0.001$) in presence of haloperidol at 90 min. However, only BNS-ET at 200 mg/kg did not exhibit any catalepsy and appeared same as the normal animals.

In vehicle treated animals, haloperidol (1 mg/kg) produced maximum catalepsy of 253.20 sec. The ASP-ME (50, 100, 200 mg/kg) significantly potentiated haloperidol induced catalepsy at each time interval (0-180 min at 30 min interval) in dose dependent manner 181.4, 287.00 and 331.6 respectively ($P < 0.001$) in presence of haloperidol at 90 min. However, only ASP-ME at 200 mg/kg did not exhibit any catalepsy and appeared same as the normal animals.

In the present study, ASL-ME, BNS-ET and ASP-ME extracts (50, 100 and 200 mg/kg) significantly potentiated haloperidol-induced catalepsy. The potentiation of catalepsy is indicative of the ability of the drug to antagonize dopamine $D_2$ receptor in striatum.

In view of antiserotonergic property of extracts, the effect of extracts was further evaluated on stress related perturbations. Chronic foot shock induced stress is widely accepted chronic mild stress model. Stressful treatments (like foot shock induced stress) have long been associated with increased activity of brain catecholamine and serotonergic neurons. A brief period of foot shock increases serum corticosterone, concentration of tryptophan, adrenaline and serotonin in several brain regions. Foot shock-induced stress not only induces behavioral depression but also causes induction of several physiological and biochemical perturbations in rats (Bhattacharya & Muruganandam, 2003).

In foot shock induced aggression study animal treated with ASL-ME, BNS-ET and ASP-ME extracts (100 and 200 mg/kg) and haloperidol (1 mg/kg) showed significant ($P < 0.05$, $P < 0.001$) as well as dose dependent decrease in number of fights. ASL-ME (100 and 200 mg/kg.) showed 23.20 and 10.20, BNS-ET (100 and 200 mg/kg.) showed 22.00 and 12.40 and ASP-ME (100 and 200 mg/kg.) 26.60 and 12.20 respectively whereas haloperidol (1 mg/kg) showed 6.00 as compared to control 52.60. While the dose 50 mg/kg of ASL-ME, BNS-ET and ASP-ME produces insignificant change in terms of above parameters. Combining results of antiaggression evaluation assist to contemplate that potential of ASL-ME, BNS-ET and ASP-ME is comparable to standard drug haloperidol.
Results of *in vitro* and *in vivo* Pharmacological screening suggest that ASL-ME, BNS-ET and ASP-ME exhibited significant and promising psychotropic and anxiolytic activity. By considering nature of obtained extracts of selected plants, for further study of fractions, only ASL-ME and BNS-ET extracts were selected.

The ASL-ME and BNS-ET was further fractionated successively by continuous hot extraction method with Petroleum-ether (60-80°C), Chloroform, Ethyl acetate and Acetone (ASL-PEF, ASL-CHF, ASL-EAF, ASL-ACF and BNS-PEF, BNS-CHF, BNS-EAF, BNS-ACF respectively) using Soxhlet extractor resulted in 0.8, 0.4, 1.9, 0.5 and 0.6, 0.4, 3.7, 29.7% yield respectively. Percent yield of ASL-EAF (1.9) and BNS-ET (29.7) was found better as compared other fractions of respective extracts.

All the fractions were screened for presence of phytoconstituents (Trease & Evans, 1983). The preliminary phytochemical screening revealed the presence of flavonoids, tannins and saponins in ASL-PEF; flavonoids and tannins in ASL-CHF; flavonoids, tannins and saponins in ASL-EAF whereas saponins in BNS-PEF; flavonoids in BNS-EAF and flavonoids, tannins, saponins and alkaloids in BNS-ACF.

Presence of phytoconstituents was ratified with the help of TLC and HPLC fingerprinting of test fractions and standardized using spectrophotometric methods. TLC fingerprinting profile of ASL-PEF, ASL-CHF, ASL-EAF, ASL-ACF and BNS-PEF, BNS-CHF, BNS-EAF, BNS-ACF shown presence of flavonoids, saponins, as well as tannins while HPLC fingerprinting confirmed presence of bioactive phytoconstituents showing same retention time as that of standard gallic acid (3.830), quercetin (5.760) and rutin (3.820), ASL-PEF shown (3.48, 3.82), ASL-CHF (3.50, 3.83), ASL-EAF (3.50, 5.26, 3.82) and BNS-PEF (3.48), BNS-EAF (3.82), BNS-ACF (3.50, 3.92) respectively while as a caffeic acid and P-hydroxy cinnamic acid were found absent in all fractions.

In addition, spectroscopic standardization provides information of quantitative analysis of phytoconstituents content. Spectrophotometric quantitative determination of the particular phytoconstituents for standardization was decided on the basis of preliminary phytochemical analysis of respective fractions. ASL-PEF found to contain 4.20 µg/mg of GAE as total polyphenols and 2.13 µg/mg of RE as a total flavonoids. ASL-CHF found to contain 0.02
μg/mg of GAE and 0.01 μg/mg of RE as total flavonoids. ASL-EAF found to contain 11.74 μg/mg of GAE, and 4.28 μg/mg of RE as total flavonoids. It suggests that ASL-EAF contains more bioactive phytoconstituents followed by ASL-PEF and ASL-CHF. BNS-ME fraction revealed that, BNS-EAF found to contain 1.24 μg/mg of GAE as total polyphenols and 0.01 μg/mg of RE as a total flavonoids. BNS-ACF found to contains 8.62 μg/mg of GAE as total polyphenols and 3.26 μg/mg of RE as a total flavonoids. It suggests that BNS-ACF contains more bioactive phytoconstituents as compared to BNS-EAF. Among ASL-ME extract, ASL-EAF fractions exhibit highest amount of total polyphenols (11.74 mg/g of GAE), and total flavonoid (4.28 mg/g of RE) while BNS-ET extract, BNS-ACF fractions exhibit highest amount of total polyphenols (8.62 mg/g of GAE) and flavonoid (3.26 mg/g of RE). Good amount of polyphenols (flavonoids as well as tannins) have been directly correlated with biological/pharmacological action.

Test fractions were further screened for antioxidant activity. Antioxidant profile (in vitro) was screened in terms of DPPH scavenging (% DPPH scavenging and % RRI of DPPH) ability (Shahidi & Wanasundara, 1992; Sanchez, et al, 1999; Soares, et al, 1997).

The percent DPPH scavenging activity possessed by standard antioxidant ascorbic acid, ASL-PEF, ASL-CHF, ASL-EAF, ASL-ACF, BNS-PEF, BNS-CHF, BNS-EAF and BNS-ACF was found to be 87.46, 29.23, 32.72, 72.13, 38.18, 36.76, 42.19, 48.28 and 82.23 % respectively at concentration 40 μg/ml. ASL-EAF and BNS-ACF exhibited better antioxidant activity than the other fractions.

Evaluation of % RRI of DPPH helps to measure the ability of test fractions to scavenge radicals at different time interval such as 0.5, 01, 03 and 07 h (Yamasaki, et al, 1994). ASL-EAF showed highest % RRI of 07.16%, followed by ASL-PEF (18.78%), ASL-CHF (22.23%) and ASL-ACF (23.64) at 07th h. BNS-ACF showed highest % RRI of 05.23% as compared to BNS-PEF (36.20%), BNS-CHF (36.96%) and BNS-EAF (22.61%) at 07th h. suggesting the ability of ASL-EAF and BNS-ACF to scavenge the free radical and protecting biological component from invading action for longer duration as compared to other fractions.
A result of *in vitro* antioxidant suggests that ASL-PEF, ASL-CHF, ASL-ACF, BNS-PEF, BNS-CHF and BNS-EAF posse’s weak anti-oxidant activity as well as also showed presence of fewer amounts of polyphenols as compared to ASL-EAF and BNS-ACF; which showed correlation between amount of polyphenols and antioxidant activity and hence excluded for further studies. ASL-EAF and BNS-ACF selected for further studies, which are supported by contents of phytoconstituents determined by spectrophotometric standardization, TLC and HPLC fingerprinting; hence ASL-EAF and BNS-ACF pooled out and further evaluated for *in vitro* and *in vivo* Pharmacological screening.

In the present study, the effect of ASL-EAF and BNS-ACF was studied on various *in vitro* preparations to assess its effect on various receptors and neurotransmitters.

Dopamine and serotonin at (100 µg/ml) log dose of 2.5 exhibited 111.40 and 84.60 of percent response while ASL-EAF at (25 mg/ml; 0.5ml) in presence of dopamine and serotonin showed 81.80 and 69.20 of percent response indicating ASL-EAF is having ability to significantly inhibit dopamine and serotonin induced contraction on isolated rat vas deferens and rat fundus respectively, whereas Ach produces 124.20 percent response, while ASL-EAF in presence of Ach potentiates response and produces 135.20 percent response. The significance was observed mainly at all log dose for dopamine and Ach whereas log dose (1.6 to 2.5) for serotonin.

Dopamine and serotonin at (100 µg/ml) log dose of 2.5 exhibited 117.40 and 105.48 of percent response while BNS-ACF at (25 mg/ml; 0.5ml) in presence of dopamine and serotonin showed 75.80 and 54.80 of percent response indicating BNS-ACF is having ability to significantly inhibit dopamine and serotonin induced contraction on isolated rat vas deferens and rat fundus respectively, whereas Ach produces 96.40 percent response, while BNS-ACF in presence of Ach potentiates response and produces 118.60 percent response. The significance was observed mainly at all log dose for dopamine and Ach whereas log dose (1.6 to 2.5) for serotonin.

The result of the *in vitro* test indicates that ASL-EAF and BNS-ACF significantly inhibited dopamine induced contractions on rat vas deferens and serotonin induced contractions on rat fundus while potentiated acetylcholine induced contractions on goat tracheal chain preparation. Thus, it is concluded that
the ASL-EAF and BNS-ACF possess antidopaminergic and antiserotonergic as well as cholinergic potentiating effect.

Based upon the in vitro results of ASL-EAF and BNS-ACF further in vivo models were selected. In the present work, the effect of ASL-EAF and BNS-ACF was studied in several behavioral animal models like elevated plus maze, light/dark paradigm for its anxiolytic property, forced swim test (FST) and tail suspension test (TST) for anti-depressant activity, PTZ induced convulsion for anticonvulsant activity, lithium sulphate induced head twitches, haloperidol induced catalepsy for antipsychotic activity and foot shock induce aggression for anti-aggressive activity.

Anxiolytic effect of ASL-EAF and BNS-ACF has been studied using elevated plus maze in terms of time spent (sec) in open arms, enclosed arms and central zone as well as entries in open arms and enclosed arms.

The animal treated with ASL-EAF (50, 75 mg/kg) and diazepam 1 mg/kg exhibited significant ($P < 0.05$, $P < 0.001$) increased in the occupancy in time spent in open arms (121.7, 162.2 and 200.0 sec) and Time spent in central zone (35.17, 42.50 and 61.83 sec) while ASL-EAF 25 mg/kg showed insignificant increased in time spent in open arms (57.33 sec) and time spent in central zone (17.33 sec) as compared to vehicle treated group rats (32.67 and 17.00 sec) respectively whereas ASL-EAF (25, 50, 75 mg/kg) and diazepam 1 mg/kg showed significant ($P < 0.05$, $P < 0.001$) decrease in the time spent in enclosed arms (217.7, 124.0, 102.5 and 50.17 sec) respectively as compared to vehicle treated groups rats (323.7 sec). The animal treated with ASL-EAF 75 mg/kg and diazepam 1 mg/kg showed significant ($P < 0.001$) increased count of entries to the open arms (25.83 and 31.67) while ASL-EAF (25, 50 mg/kg) showed insignificant increased in count of entries to the open arms (15.00, 20.33) respectively as compared to vehicle treated group rats (6.00) whereas animal treated with ASL-EAF (25, 50, 75 mg/kg) and diazepam 1 mg/kg showed significant ($P < 0.05$, $P < 0.001$) decreased count of entries to enclosed arms (21.33, 12.83, 11.50 and 9.66) respectively as compared to vehicle treated group rats 35.33.

The vehicle treated group rats spent 32.67 sec in open arms and 323.70 sec in enclosed arms while BNS-ACF (50 and 75 mg/kg) and diazepam (1 mg/kg) showed significant ($P < 0.001$) increase in the occupancy in the open
arms (96.50, 173.00 and 200.0 sec) and decrease in enclosed arms (157.70, 103.80 and 50.17 sec) while BNS-ACF 25 mg/kg showed insignificant increase in open arms (45.50 sec) and enclosed arms (253.20 sec) respectively. The vehicle treated group rats spent 17.00 sec in central zone while BNS-ACF 75 mg/kg and diazepam 1 mg/kg showed significant ($P < 0.001$) increase in the occupancy in central zone (45.67 and 61.83 sec) respectively and BNS-ACF (25, 50 mg/kg) showed insignificant increased in central zone (22.00, 29.33 sec) respectively. The animal treated with BNS-ACF 75 mg/kg and diazepam 1 mg/kg exhibited significant ($p < 0.001$) increased count of entries to open arms 30.33 and 31.67 respectively while BNS-ACF (25, 50 mg/kg) showed insignificant increased count of entries to open arms (21.33 and 9.83) respectively as compared to vehicle treated group rats (6.00) whereas BNS-ACF (50, 75 mg/kg) and diazepam (1 mg/kg) showed significant ($p < 0.001$) decreased count of entries to enclosed arms (14.83, 8.00 and 9.66) respectively while BNS-ACF 25 mg/kg showed insignificant decreased count of entries to enclosed arms 27.00 as compared to vehicle treated group rats 35.33.

In EPM test, ASL-EAF and BNS-ACF increased the exploration and the time spent in open arm. The number of entries and time spent in the enclosed arms were reduced by ASL-EAF and BNS-ACF (50, 75 mg/kg), in comparison to control values. The anxiolytic effect of the fractions was more prominent at 75 mg/kg respectively. In this study, diazepam treated animals increased the number of open arm entries, reducing the natural animal’s aversion to the open arms and promoting the exploration thereof, indicating an anxiolytic effect.

Anxiolytic activity of ASL-EAF and BNS-ACF has also been evaluated using light dark model. The animals treated with ASL-EAF (50 and 75 mg/kg) and diazepam (1 mg/kg) showed significant ($P < 0.001$) increase in time spent in light zone (122.8, 138.8 and 234), number of crossing (27.50, 51.67 and 60.83), transfer latency (22.17, 38.83 and 57.50) respectively as compared to control (17.17, 2.50, 12.17), while significant decrease in animal treated with all fractions in time spent in dark zone (140.20, 132.80, 89.83 and 79.00) was observed as compared to control (217.5). The dose 25 mg/kg of ASL-EAF did not produce any significant change except time spent in dark zone parameters.

The animals treated with BNS-ACF (25, 50 and 75 mg/kg) and diazepam (1 mg/kg) showed significant ($P < 0.05$ and $P < 0.001$) increase in time spent in
light zone (58.50, 70.00, 108.5 and 234.00 sec) as compared to vehicle treated group rats (17.17 sec). The animal treated with BNS-ACF 75 mg/kg and diazepam 1 mg/kg exhibited significantly ($P < 0.001$) decreased in time spent in dark zone (142.70 and 79.00 sec) respectively while BNS-ACF (25, 50 mg/kg) showed insignificant decreased in time spent in dark zone (184.50, 178.50 sec) as compared to vehicle treated group rats (217.5 sec) whereas animal treated with BNS-ACF (50, 75 mg/kg) and diazepam 1 mg/kg showed significant ($P < 0.05$ and $P < 0.001$) increased in number of crossing (21.50, 37.33 and 60.83) and in transfer latency (27.00, 50.67 and 57.50) respectively while BNS-ACF 25 mg/kg showed insignificant increased in number of crossing (17.17) and transfer latency (12.50) as compared to vehicle treated group rats (2.50, 12.17) respectively.

In this model, ASL-EAF and BNS-ACF produced significant increase in time spent in lit box as compared to vehicle, showing an anxiolytic activity. Combining results of all the anxiolytic evaluation assist to contemplate the anxiolytic action of ASL-EAF and BNS-ACF is comparable to standard drug diazepam.

The anti-depressant effect of ASL-EAF and BNS-ACF was evaluated by using forced swim test (FST) and tail suspension test (TST).

Animals treated with ASL-EAF, BNS-ACF (75 mg/kg) and imipramine (15 mg/kg) significantly ($P < 0.001$) decreased duration of immobility in tail suspension test (44.83, 45.00 and 43.17) respectively and in force swim test (22.83, 22.83 and 18.83 respectively while ASL-EAF, BNS-ACF (25, 50 mg/kg) insignificantly decreased (116.80, 94.83), (139.0, 99.33) respectively as compared to control (143.70 and 128.83).

Animal treated with ASL-EAF, BNS-ACF (50, 75 mg/kg) and imipramine (15 mg/kg) significantly ($P < 0.05$, $P < 0.001$) increased latency of immobility in tail suspension test (55.83, 88.50), (67.67, 93.17) and (113.20) respectively and ASL-EAF, BNS-ACF 25 mg/kg showed insignificant increased (14.83, 36.50) as compared to control (113.20) while in force swim test animal treated with ASL-EAF, BNS-ACF (75 mg/kg) and imipramine (15 mg/kg) showed significantly ($P < 0.001$) increased latency of immobility (45.50, 52.83 and 57.56) respectively whereas ASL-EAF and BNS-ACF (25, 50 mg/kg) showed insignificant increased (15.00, 22.33) and (11.50, 23.33) respectively as compared to control (7.83).
Combining results of evaluation assist to contemplate the antidepressant action of ASL-EAF and BNS-ACF is comparable to standard drug diazepam.

Anticonvulsant activity of ASL-EAF and BNS-ACF has also been evaluated using PTZ induced convulsion model.

The animal treated with PTZ (80 mg/kg) induced myoclonic (50.20) and clonic spasm (48.40) in 100% of control mice, Pretreatment with ASL-EAF (50 and 75 mg/kg) and diazepam (1 mg/kg) significantly ($P < 0.001$) delayed the onset initially in myoclonic (132.80, 167.40 and 286.6) and then in clonic spasms (199.40, 255.20 and 466.2) respectively. Pretreatment with BNS-ACF (50 and 75 mg/kg) and diazepam (1 mg/kg) significantly ($P < 0.001$) delayed the onset initially in myoclonic (129.40, 162.40 and 286.6) and then in clonic spasms (146.20, 187.20 and 466.2) respectively. In addition, percentage of animal showing mortality was decreased and 100% mortality was observed in control group. ASL-EAF and BNS-ACF delayed occurrence of seizures and reduced mortality indicating its anticonvulsant potential.

Antipsychotic activity of ASL-EAF and BNS-ACF has been evaluated using lithium sulphate induced head twitches and haloperidol induced catalepsy models.

In present study the vehicle treated group rats, Lithium sulphate produced 51.17 number of head twitches whereas ASL-EAF (25, 50 and 75 mg/kg) and BNS-ACF (50, 75 mg/kg) showed significantly ($P < 0.05, P < 0.001$) decreased number of head twitches to (33.50, 28.17 and 12.33) and (25.50 and 14.67) respectively. Ondansetron (a 5HT$_3$ antagonist) also reduced the number of head twitches (7.83) showing its effect on serotonergic system.

The results showed that at 25, 50 and 75 mg/kg and 50, 75 mg/kg dose of ASL-EAF and BNS-ACF significantly decreased lithium-induced head twitches. The fractions also inhibited contractions induced by serotonin on rat fundus. Thus, suggesting possible inhibitory effect of ASL-EAF and BNS-ACF on serotonergic system.

In vehicle treated animals, haloperidol (1 mg/kg) produced maximum catalepsy (224.60±7.40) after 90 min. The ASL-EAF (25, 50, 75 mg/kg) significantly potentiated haloperidol induced catalepsy at each time interval (0-180 min at 30 min interval) in dose dependent manner. ASL-EAF at dose 25 and 50 mg/kg showed maximum cataleptic score 263.40±10.94 and 308.20±6.68
respectively at 120 min ($P < 0.001$) in haloperidol treated animals whereas 75 mg/kg showed 317.60±6.65 at 90 min ($P < 0.001$). However, only ASL-EAF at 75 mg/kg did not exhibit any catalepsy and appeared same as the normal animals.

In vehicle treated animals, haloperidol (1 mg/kg) produced maximum catalepsy (218.60±10.71) at 120 min. The BNS-ACF (25, 50, 75 mg/kg) significantly potentiated haloperidol induced catalepsy at each time interval (0-180 min at 30 min interval) in dose dependent manner. BNS-ACF at dose 25, 50 and 75 mg/kg showed maximum cataleptic score 263.00±6.48, 301.20±7.13 and 331.00±11.57 respectively at 90 min ($P < 0.001$) in haloperidol treated animals; whereas BNS-ACF 75 mg/kg did not exhibit any catalepsy and appeared same as the normal animals.

In the present study, ASL-EAF and BNS-ACF (25, 50, and 75 mg/kg) significantly potentiated haloperidol-induced catalepsy. The potentiation of catalepsy is indicative of the ability of the drug to antagonize dopamine D$_2$ receptor in striatum.

In view of antiserotonergic property of fractions, the effect of fractions was further evaluated on stress related perturbations. Chronic foot shock induced stress is widely accepted chronic mild stress model. Stressful treatments (like foot shock induced stress) have long been associated with increased activity of brain catecholamine and serotonergic neurons. A brief period of foot shock increases serum corticosterone, concentration of tryptophan, adrenaline and serotonin in several brain regions. Foot shock-induced stress not only induces behavioral depression but also causes induction of several physiological and biochemical perturbations in rats (Bhattacharya & Muruganandam, 2003).

Animal treated with ASL-EAF, BNS-ACF (50, 75 mg/kg) and Haloperidol (1 mg/kg) (26.00, 11.33), (29.83, 14.17) and (6.00) showed significantly ($P < 0.001$) dose dependent decrease in number of fight while 25 mg/kg showed insignificant decrease (43.17, 45.83) in foot shock-induced aggression as compared with control (50.83). Further ASL-EAF, BNS-ACF (25, 50, 75 mg/kg) and Haloperidol (1 mg/kg) (67.50, 85.83, 107.50), (63.00, 100.30, 139.20) and (254.70) showed significantly ($P < 0.001$) increased latency to fight as compared to control (16.50). Combining results of antiaggression evaluation assist to contemplate that potential of ASL-EAF and BNS-ACF is comparable to standard drug haloperidol.