Neuropharmacology also involves understanding the actions of drugs on the functions of the brain, whether it is on single cells or behavior, is a multilevel, multifaceted process that begins with and builds upon the concept of molecular interactions. Neuroprotective are the agents, which are used for treatment of neurodegenerative disorder or neural cell damage.

In today’s life, stress, strain, sedentary life style, obesity, lack of physical work and environmental pollution are becoming crucial factors in the genesis and progression of variety of CNS diseases and disorders which goes undiagnosed and untreated in many patients. Synthetic drugs available for treatment of anxiety and other mental illnesses have various adverse effects. The several medicinal plants identified and mentioned by traditional system as being useful for treating CNS disorders. A traditional medical system has been mostly focused on its plant based remedies, with the underlying understanding that some species may contain therapeutically useful compounds. However as the screening target mechanisms of many traditional medicines mostly remained unclear and exclusive and needed to be evaluated at the extent of scientifically acceptable/mechanism based approach.

The present work was aimed to evaluate the *Amaranthus spinosus* leaves extracts, *Annona squamosa* fruits pulps extracts and *Brassica nigra* seeds extract for neuroprotective activity. As these plants have been ethno pharmacologically claimed for the treatment of various disorders. Moreover, *Amaranthus spinosus* leaves extract have been reported for the presence of polyphenols (flavonoids as well as tannins) and triterpenoids. *Annona squamosa* fruits pulps have been found to contain polyphenols (flavonoids as well as tannins), while *Brassica nigra* seeds showed presence of polyphenols (flavonoids as well as tannins), fixed oil, saponins and triterpenoids. Phytochemical and ethno pharmacological claims prompted us to carry out and correlate the phyto-pharmacological evaluation of selected plants part for neuroprotective activity.

Phytochemical evaluation of extracts from selected plants includes preliminary phytochemical screening, TLC, HPLC fingerprinting and spectrophotometric standardization of extracts for the quantitative evaluation for
Contents of total flavonoids and total polyphenols. Pharmacological screening of all the extracts was evaluated in terms of in vitro and in vivo activity. In vitro assays highlighted antioxidant, dopamine-induced contractions of isolated rat vas deferens, serotonin-induced contractions of isolated rat fundus and acetylcholine-induced contractions of isolated goat tracheal chain preparation. In vivo evaluation methods encompass anxiolytic, antidepressant, anticonvulsant, antipsychotic and anti-aggressive activity models.

_A. spinosus_ leaves were extracted successively with pet ether (60-80) (ASL-PE), chloroform (ASL-CH), acetone (ASL-AC) and methanol (ASL-ME). _B. nigra_ seeds were extracted with pet ether (60-80) (BNS-PE), chloroform (BNS-CH), acetone (BNS-AC) and ethanol (BNS-ET). While _A. squamosa_ fruit pulp was extracted with methanol (ASP-ME) using maceration process. According to % yield, the order of extract is ASP-ME>BNS-PE>BNS-ET.ASL-ME>ASL-AC>ASL-CH>ASL-PE>BNS-CH>BNS-AC (Table 4.4)

All the extracts were screened qualitatively for phytochemical constituents and found to contain polyphenols, saponins, steroids, triterpenoids (Table 4.5) and presence of these phytoconstituents in extracts was ratified with the help of TLC and HPLC fingerprinting (Table 4.6, 4.11). Moreover, extracts were standardized for the contents of phytoconstituents such as total flavonoids and total polyphenols as a part of quality standard parameters (Table 4.9).

Antioxidant potential of test extracts was screened with the aid of DPPH scavenging and percent residual rate of inhibition (% RRI) of DPPH and compared with standard antioxidant ascorbic acid (Table 4.12).

Extracts which revealed weak antioxidant potential were pooled out and eliminated for further pharmacological assays. Based on antioxidant results ASL-PE, ASL-CH, ASL-AC, BNS-PE, BNS-CH and BNS-AC were not considered for further evaluation. This was also supported by less content of phytoconstituents such as flavonoids and tannins as compared to other extracts which can be correlated as a part of selection of potent extracts. Therefore, ASL-ME, BNS-ET and ASP-ME were screened further. Extracts with comparatively better antioxidant potential were further evaluated for acute and sub-acute toxicity, in vitro and in vivo pharmacological screening.

ASL-ME, BNS-ET and ASP-ME were also further evaluated for safety margin for oral administration as per OECD test (guideline no 423 acute toxicity
class method). ASL-ME, BNS-ET and ASP-ME were found to be safe as observed by lack of clinical toxicity symptoms and mortality (LD<sub>50</sub> > 2000 mg/kg). Therefore dose of 50, 100 and 200 mg/kg; p. o. was selected as a safe dose for the <i>in vitro</i> viz. dopamine-induced contractions of isolated rat vas deferens, serotonin-induced contractions of isolated rat fundus and acetylcholine-induced contractions of isolated goat tracheal chain preparation and <i>in vivo</i> activity viz. anxiolytic, antidepressant, anticonvulsant, antipsychotic and anti-aggressive activity of plant extracts (Table 4.13).

The effect of ASL-ME, BNS-ET and ASP-ME was studied on various <i>in vitro</i> preparations to assess its effect on various receptors and neurotransmitters. The result of the <i>in vitro</i> test indicates that ASL-ME, BNS-ET, and ASP-ME inhibited dopamine induced contractions on rat vas deferens and significantly inhibited serotonin-induced contractions on rat fundus while potentiated acetylcholine-induced contractions on goat tracheal chain preparation. Thus, it is concluded that the ASL-ME, BNS-ET and ASP-ME possess antidopaminergic and antiserotonergic activity whereas potentiated cholinergic transmission (Table 4.14, 4.15, 4.16).

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, also been evaluated using light dark model viz. time spent in light compartment, time spent in dark compartment, number of crossings between these two compartments and transfer latency (Table 4.17, 4.18, 4.19).

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.
In light and dark 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.

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).

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 (Table 4.20).

Anticonvulsant activity of ASL-ME, BNS-ET and ASP-ME has also been evaluated using PTZ induced convulsion model.

ASL-ME, BNS-ET and ASP-ME delayed occurrence of seizures and reduced mortality indicating its anticonvulsant potential (Table 4.21). Diazepam, a GABA<sub>A</sub> 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.

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

In lithium sulphate induced head twitches 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 (Table 4.22).

In haloperidol induced catalepsy model, ASL-ME, BNS-ET and ASP-ME extracts (50, 100 and 200 mg/kg) significantly potentiated haloperidol-induced catalepsy (Table 4.23, 4.24, 4.25). The potentiation of catalepsy is indicative of the ability of the drug to antagonize dopamine D<sub>2</sub> receptor in striatum.
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 as compared to control. While the dose 50 mg/kg of ASL-ME, BNS-ET and ASP-ME produces insignificant change in terms of above parameters (Table 4.26).

The ASL-ME and BNS-ET was further fractionated successively by continuous hot extraction method with Petroleum-ether (60-80°C) (ASL-PEF, BNS-PEF), Chloroform (ASL-CHF, BNS-CHF), Ethyl acetate (ASL-EAF, BNS-EAF) and Acetone (ASL-ACF, BNS-ACF) using Soxhlet Extractor. According to % yield, the order of fractions is BNS-ACF>BNS-EAF>ASL-EAF>ASL-PEF>BNS-PEF>ASL-ACF>ASL-CHF and BNS-CHF (Table 4.27).

All the fractions were screened qualitatively for phytochemical constituents and found to contain polyphenols, saponins (Table 4.28) and presence of these phytoconstituents in fractions was ratified with the help of TLC and HPLC fingerprinting (Table 4.29, 4.31). Moreover, fractions were standardized for the contents of phytoconstituents such as total flavonoids and total polyphenols as a part of quality standard parameters (Table 4.30).

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 (Table 4.32).

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.
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 (Table 4.33, 4.34, 4.35).

Based upon the *in vitro* results of ASL-EAF and BNS-ACF further *in vivo* models were selected.

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, also been evaluated using light dark model *viz.* time spent in light compartment, time spent in dark compartment, number of crossings between these two compartments and transfer latency.

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 arm and promoting the exploration thereof, indicating an anxiolytic effect (Table 4.36).

In light and dark model, ASL-EAF and BNS-ACF produced significant increase in time spent in lit box as compared to vehicle, showing an anxiolytic activity (Table 4.37).

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 and force swim test while ASL-EAF, BNS-ACF (25, 50 mg/kg) insignificantly decreased as compared to control.
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 and ASL-EAF, BNS-ACF 25 mg/kg showed insignificant increased as compared to control 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 whereas ASL-EAF and BNS-ACF showed insignificant increased as compared to control (Table 4.38).

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 and clonic spasm 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 and then in clonic spasms. Pretreatment with BNS-ACF (50 and 75 mg/kg) and diazepam (1 mg/kg) significantly \((P < 0.001)\) delayed the onset initially in myoclonic and then in clonic spasms. In addition, percentage of animal showing mortality was decreased and 100% mortality was observed in control group (Table 4.39).

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 model.

In lithium sulphate induced head twitches, 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 (Table 4.40).

In haloperidol induced catalepsy model, 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\textsubscript{2} receptor in striatum (Table 4.41, 4.42, 4.43).

In foot shock induced aggression study animal treated with ASL-EAF, BNS-ACF (50, 75 mg/kg) and Haloperidol (1 mg/kg) showed significantly ($P < 0.001$) dose dependent decrease in number of fight while 25 mg/kg showed insignificant decrease in foot shock-induced aggression as compared with control. Further ASL-EAF, BNS-ACF and Haloperidol (1 mg/kg) showed significantly ($P < 0.001$) increased latency to fight as compared to control (Table 4.44).

Combining results of antiaggression evaluation assist to contemplate that potential of ASL-EAF and BNS-ACF is comparable to standard drug haloperidol.