

**EVALUATION OF ANTICONVULSANT AND ANXIOLYTIC
ACTIVITY OF METHANOLIC EXTRACT OF LEAVES OF
SYZYGIUM AQUEUM (Brum. f)**

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**Submitted By
Mrs. GEETHU KRISHNA. O.**

Reg No: 261525707

Under the guidance of

**Mr. G. THAMOTHARAN, M. Pharm.,
Assistant Professor**



**DEPARTMENT OF PHARMACOLOGY
JKKMMRF'S - ANNAI JKK SAMPOORNI AMMAL
COLLEGE OF PHARMACY,
KOMARAPALAYAM – 638 183
TAMIL NADU**

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CHAPTER 1

INTRODUCTION

1.1. INTRODUCTION TO HERBAL MEDICINE

For centuries people have used plants for healing. Plant products as parts of foods or botanical potions and powders have been used with varying success to cure and prevent diseases throughout history. The strong historic bond between plants and human health began to unwind in 1897, when Friedrich Bayer and Co. introduced synthetic acetyl salicylic acid (aspirin) to the world. Aspirin is a safer synthetic analogue of salicylic acid, an active ingredient of willow bark, and was discovered independently by residents of both the New and Old worlds as a remedy for aches and fevers (Ilyaraskin and David M. Ribnicky, 2002).

Herbal medicine is the use of plants, plant parts, their water or solvent extracts, essential oils, gums, resins, exudates or other form of advanced products made from plant parts used therapeutically to provide proactive support of various physiological systems; or, in a more conventional medical sense, to treat, cure, or prevent a disease in animals or humans (Weiss RF & Fintelmann V. *et al*,2000).

About 70-80% of the world populations, particularly in the developing countries, rely on non-conventional medicine in their primary healthcare as reported by the World Health Organization (Akerele O *et al.*, 1993).

In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants (Vulto AG & Smet PAGM *et al.*, 1988). This interest in drugs of plant origin is due to several reasons, namely, conventional medicine can be inefficient (e.g. side effects

and ineffective therapy), abusive and/or incorrect use of synthetic drugs results in side effects and other problems, a large percentage of the world, conventional pharmacological treatment, and folk medicine and ecological awareness suggest that “natural” products are harmless.

About 25% of the drugs prescribed worldwide come from plants, 121 such active compounds being in current use. Of the 252 drugs considered as basic and essential by the World Health Organization (WHO, 1991), 11% are exclusively of plant origin and a significant number are synthetic drugs obtained from natural precursors.

Examples of important drugs obtained from plants are digoxin from *Digitalis* species, quinine and quinidine from *Cinchona* spp., vincristine and vinblastine from *Catharanthus roseus*, atropine from *Atropa belladonna* and morphine and codeine from *Papaver somniferum* (Rates SMK *et al.*, 2001).

About 500 plants with medicinal use are mentioned in ancient literature and around 800 plants have been used in indigenous systems of medicine. India is a vast repository of medicinal plants that are used in traditional medical treatments (Chopra *et al.*, 1956).

The various indigenous systems such as Siddha, Ayurveda, Unani and Allopathy use several plant species to treat different ailments (Rabe and Staden, 1997). Herbal medicines as the major remedy in traditional system of medicine have been used in medical practices since antiquity. The practices continue today because of its biomedical benefits as well as place in cultural beliefs in many parts of world and have made a great contribution towards maintaining human health.

1.1.1. The role of herbal medicines in traditional healing

The World Health Organization (WHO) has recently defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today (WHO,1991). Or say, traditional medicine is the synthesis of therapeutic experience of generations of practicing physicians of indigenous systems of medicine.

The traditional preparations comprise medicinal plants, minerals, organic matter, etc. Herbal drugs constitute only those traditional medicines which primarily use medicinal plant preparations for therapy. The earliest recorded evidence of their use in Indian, Chinese Egyptian, Greek, Roman and Syrian texts dates back to about 5000 years.

The classical Indian texts include *Rigveda*, *Athurveda*, *Charak Samhita* and *Sushruta Samhita*. The herbal medicines/traditional medicaments have, therefore, been derived from rich traditions of ancient civilizations and scientific heritage.

1.1.2. Herbal medicine in India

India is one of the 12 mega biodiversity centers having over 45,000 plant species. Its diversity is unmatched due to the presence of 16 different agroclimatic zones, 10 vegetative zones and 15 biotic provinces. The country has 15,000–18,000 flowering plants, 23,000 fungi, 2500 algae, 1600 lichens, 1800 bryophytes and 30 million micro-organisms (Drugs and Pharmaceuticals, 1998).

India also has equivalent to 3/4 of its land exclusive economic zone in the ocean harbouring a large variety of flora and fauna, many of them with therapeutic

properties. About 1500 plants with medicinal uses are mentioned in ancient texts and around 800 plants have been used in traditional medicine.

1.1.3. Difference of Herbal and Conventional Drugs

Compared with well-defined synthetic drugs, herbal medicines exhibit some marked differences, namely:

- The active principles are frequently unknown
- Standardization, stability and quality control are feasible but not easy;
- The availability and quality of raw materials are frequently problematic;
- Well-controlled double-blind clinical and toxicological studies to prove their efficacy and safety are rare;
- Empirical use in folk medicine is a very important characteristic;
- They have a wide range of therapeutic use and are suitable for chronic treatments;
- The Occurrence of undesirable side effects seems to be less frequent with herbal Medicines, but well-controlled randomized clinical trials have revealed that they also exist;
- They usually cost less than synthetic drugs (Calixto J B *et al.*, 2000).

1.1.4. Relationship between Ayurveda and modern medicine

Ayurveda, one of the major traditional forms of medical practice in India, has produced many useful leads in developing medications for chronic diseases.

Almost 25 centuries ago, Hippocrates proclaimed, Let food be the medicine and medicine be the food. (David J. Newman & Gordon M. Cragg, 2003).

Combining the strengths of the knowledge base of traditional systems such as Ayurveda with the dramatic power of combinatorial sciences and High Throughput Screening will help in the generation of structure- activity libraries. Ayurvedic knowledge and experiential database can provide new functional leads to reduce time, money and toxicity –the three main hurdles in drug development. These records are particularly valuable, since effectively these medicines have been tested for thousands of years on people. Efforts are underway to establish pharmaco epidemiological evidence base regarding safety and practice of ayurvedic medicines.

1.1.5. Herbal medicine standardization

In indigenous/traditional systems of medicine, the drugs are primarily dispensed as water decoction or ethanolic extract. Fresh plant parts, juice or crude powder are a rarity rather than a rule. Thus medicinal plant parts should be authentic and free from harmful materials like pesticides, heavy metals, microbial or radioactive contamination, etc. The medicinal plant is subjected to a single solvent extraction once or repeatedly, or water decoction or as described in ancient texts.

The extract should then be checked for indicated biological activity in an experimental animal model(s).The bioactive extract should be standardized on the basis of active principle or major compound(s) along with fingerprints.

The next important step is stabilization of the bioactive extract with a minimum shelf-life of over a year. The stabilized bioactive extract should undergo

regulatory or limited safety studies in animals. Determination of the probable mode of action will explain the therapeutic profile.

The safe and stable herbal extract may be marketed if its therapeutic use is well documented in indigenous systems of medicine, as also viewed by WHO. A limited clinical trial to establish its therapeutic potential would promote clinical use. The herbal medicines developed in this mode should be dispensed as prescription drugs or even OTC products depending upon disease consideration and under no circumstances as health foods or nutraceuticals. (V.P.Kamboj *et al.*, 2000).

1.1.6. Pharmacological Actions of Herbal Medicine

1.1.6.1. Anti-inflammatory activity

The extract of *Achillea millefolium*, *Artemisia vulgaris*, *Bauhinia tarapotensis*, *Curcuma longa*, *Forsythia suspense*, *Houttuynia cordata*, *Glycyrrhiza uralensis*, *Lonicera japonica*, *Ruta graveolens*, *Securidaca longipedunculata* and *Valeriana wallichii* have shown anti-inflammatory activity.

1.1.6.2. Antidiabetic activity

From earliest period, people have using herbal plants as home remedies for the treatment of diabetics. A variety of herbal plants with antidiabetic activity are *Abroma augusta*, *Acacia melanoxylon*, *Acacia modesta*, *Acacia nilotica*, *Aconitum ferox*, *Adathoda vasika*, *Adiantum capillus*, *Adiantum incisum*, *Agrimonia eupatoria*, *Allium sativum*, *Aloe barbadensis*, *Althea officinalis*, *Apium grroveolens*, *Commiphora abyssinca*, *Embilica officinalis*, *Eucalyptus globules*, *Ginseng panax*, *Gymnema sylvestre*, *Inula helenium*, *Juniperus commmunis*, *Medicago sativa*, *Nigella sativa*, *Orthosiphon stamineus*, *Panax quinquefolius*, *Polygala senega*,

Plantago ovata, Punica granatum, Salvia officinalis, Tanacetum vulgare, Tecoma stans, Turnera diffusa.

1.1.6.3. Analgesic activity

The extracts of *Bougainvillea spectabilis, Chelidonium majus, Ficus glomerata, Dalbergia lanceolaria, Glaucium grandiflorum, Glaucium paucilobum, Nepta italic, polyalthia longifolia, Sida acuta, Toona ciliate, Zataria multiflora* and *Zingiber zerumbet* are used as analgesic agents.

1.1.6.4. Anticancer activity

Medicinal plant products exhibiting anticancer activity continue to be the subject of extensive research aimed at the development of drugs for treatment of different human tumors. The medicinal plants used for the the treatment of cancer are, *Celastrus paniculatus, Embelica ribes, Ficus glomerata, Ficus racemose, Ocimum basilicum, Plumbagozeylanica, Terminalia chebula, Tylophora indic, Wrighti tinctoria.* The extract used for treatment of breast cancer are *Buthus martensi, Colla cornu, Herb epimedii, Fructus lycii, Radix angelicae, Rhizoma corydalis, Rhizoma curculigins, Radix paeoniae, Squama manitis, Tuber curcumae.*

1.1.6.5. Anti-ageing activity

Cell membranes are particularly susceptible for to the hostility of free radicals. When nucleus is injured, the cell loses its ability to replicate itself. The impaired cell replication results in destabilized immune system, skin aging and many age related disorders. Various antioxidants neutralize the free radicals and prevent oxidation on a cellular level. The most effectual antioxidants include pine bark extract, grape seed extract and blue berries were effectual against hostility of

free radicals. Some commonly used herbs as anti-ageing agents are *Allium sativum*, *Arnica montana*, *Cucumis sativum*, *Curcuma longa*, *Ficus bengalensis*, *Lycium barbarum*, *Ocimum sanctum*, *Panax ginseng*, *Prunus amygdalis*, *Santalum album*, *Rosa damascene* and *Withania somnifera*.

1.1.6.6. Antifertility activity

Plant drugs have involved in the concentration of many scientists as a primary source of naturally occurring fertility regulating agents because of their little or no side effects. The plant that have been reported to have anti-fertility activity are *Amaranthus retroflexus*, *Artrabotrys odoratissimus*, *Barberis vulgaris*, *Carica papaya*, *Evodia rutacapra*, *Fatsia horrid*, *Hibiscus rosasinensis*, *Lornicera ciliosa*, *Pisum sativum*, *Raphanus sativus*, *Taxus baccata* and *Verbena officinalis*.

1.1.6.7. Anti-psoriasis activity

A variety of natural proprietary formulas and preparations containing plant materials have been used to provide symptomatic relief in psoriasis. The different herbal remedies for psoriasis are turmeric, curcumin, shark cartilage extract, oregano oil, milk thistle. Various antimicrobial agents like *Azardirecta indica*, *Calendula officinalis*, *Cassia tora*, *Wrightia tinctoria* have been used in the treatment of psoriasis.

1.1.6.8. Antidepressant activity

A number of nutritional and herbal supplements have shown promise for alternative treatment for depression. A large number of plants have potential function to treat depression which are described as *Bacopa monniera*, *Pana*

quinquefolius, *Piper methysticum*, *Rhodiola rosea*, *Valeriana officinalis* and *Hypericum perforatum*.

1.1.6.9. Anti-vitiligo activity

Anti-vitiligo oil is a herbal remedy manufactured with potent herbs and is produced with traditional methods and is also a complete traditional formulation. The plants which can be used in the treatment of vitiligo are *Acorus calamus*, *Adiantum capillus*, *Boswellia serrata*, *Cassia angustifolia*, *Cassia tora*, *Cinnamon cassia*, *Fumaria officinalis*, *Glycerrhiza glabra*, *Psoralea cordyfolia*, *Vitis vinifera*, *Zingiber officinale* and *Zizyphus sativa*.

1.1.7. Phyto-constituents and It's Actions

1.1.7.1. Alkaloids:

Alkaloids have derived from plant sources, they are basic, they contain one or more nitrogen atoms (usually in heterocyclic ring) and they usually have marked physiological actions on man or other animals.

Alkaloids are mainly used as antitumor (vincristine and vinblastine), anticholinergic (atropine), stimulant (caffeine), antimalarial and antipyretic (quinine), cough medicine and analgesic (codeine) etc...

1.1.7.2. Flavonoids:

Flavonoids are the largest class of polyphenols. Chemically they may be defined as a group of polyphenolic compounds consisting of substances that have two substituted benzene rings connected by the chain of three carbon atoms and an oxygen bridge.

Flavonoids possess antibacterial, anticancer, anti-inflammatory actions and used in the treatment of cardiovascular diseases.

1.1.7.3. Glycosides:

Glycosides may be defined in general as the organic compounds from plants or animal sources which on enzymatic or acid hydrolysis give one or more sugar (glycon) moieties along with non-sugar (aglycon) moiety.

Glycosides are used as cardio tonic, purgative, anti -rheumatic, analgesic, demulcent etc...

1.1.7.4. Tannins:

Tannins are chemically defined as the mixture of complex organic substances where in polyphenols are present with O-dihydroxy or O-trihydroxy groups on a phenyl ring.

Tannins are used as mild antiseptic, in the treatment of diarrhea and to forestall minor hemorrhages.

1.1.7.5. Carbohydrates:

Carbohydrate may be defined as polyhydroxy aldehydes or polyhydroxy ketones or compounds which produce them on hydrolysis, Carbohydrates are mainly used as demulscent, laxative, antidiarrhoeal, pharmaceutical agents etc...

1.1.7.6. Fixed oils and fats:

These are reserved food materials of plants and animals. Those which are liquid at 15.5°C to 16.5°C are called as fixed oils; while those which are solid or semisolid at above temperature are called as fats.

1.1.7.7. Muscilage:

These are polysaccharide complexes of sugar and amino acids, usually formed from the cell wall. They are insoluble in alcohol but swell or dissolve in water.

They are used as emulsifiers, suspending agent, demulsent etc...

1.1.7.8. Proteins and amino acids:

Proteins are complex nitrogenous organic substances of plant and animal origin. They are of great importance in the functioning of living cells. They contain carbon, hydrogen, nitrogen, oxygen and rarely Sulphur. The ultimate product of complete hydrolysis of proteins, either by chemical reagents or enzymes are amino acids.

Amino acids are group of organic compounds containing two functional groups- amino and carboxyl. The amino group is basic while the carboxyl group is acidic in nature.

Proteins are mainly used as digestant, anti-inflammatory agent, anti-coagulant, nutritive and dietary supplements. (Kokate *et al.*, 2012).

1.2. INTRODUCTION TO CENTRAL NERVOUS SYSTEM: NEURO-TRANSMISSION AND DISORDERS

Central nervous system (CNS) is functionally very complex than any other systems in the body as the relationship between the behavior of individual cell and that of the whole organ is less direct. CNS includes brain and spinal cord. Brain is an array of interrelated neural systems that regulate their own and each other's activity through intercellular chemical transmission.

1.2.1. Neurochemical Transmission in CNS:

Four processes occur in relation to nerve transmission in CNS neurotransmission, neuromodulation, neuromediation and mediation through neurotropic factors. Analogously such chemical secretions are called neurotransmitters, neuromodulators, neuromediators and neurotropic factors respective.

1.2.1.1. Neurotransmitters:

Synthesized in presynaptic neurons and are released into synaptic cleft to rapidly stimulate or inhibit postsynaptic neurons.

Eg: Acetylcholine, dopamine, norepinephrine (epinephrine in reticular formation), 5-hydroxytryptamine, gamma-amino butyric acid, glycine, glutamate, aspartate, endogenous opioids, cholecystokinin, tachykinins etc...

1.2.1.2. Neuromodulators:

They are released by neurons and astrocytes to produce slower pre-or postsynaptic responses. Neuromodulation generally relates to synaptic plasticity that

means long-term changes in synaptic transmission, connectivity and efficacy following pathological damage (as in epilepsy or drug dependence) or following physiological alterations in neuronal activity (as in learning and memory).

eg. Carbon dioxide, locally released adenosine, some purines, peptides, prostaglandins, arachidonic acid metabolites and Nitric oxide.

1.2.1.3. Neuromediators:

They are second messengers that play crucial role in elicitation of postsynaptic responses produced by neurotransmitters. eg. cAMP, cGMP and inositol phosphate.

1.2.1.4. Neurotropic factors:

They are mainly released by CNS neurons, astrocytes and microglia and act longer than neuromodulators to regulate the growth and morphology of neurons and control long-term changes in brain (synaptic plasticity, remodeling, phenotype characteristics) mainly by affecting gene transcription by acting through tyrosine kinase-linked receptors. eg. Cytokines, chemokines, growth factors.

1.2.2. CNS Neurotransmitters and their receptors

1.2.2.1. Amino Acids:

The CNS contains high concentrations of certain amino acids mainly, glutamate and gamma-amino butyric acid (GABA). The dicarboxylic acids (glutamate and aspartate) produce excitation and monocarboxylic ω -amino acids (GABA, glycine, β -alanine and taurine) produce inhibition.

GABA and its receptors:

GABA is the major inhibitory neurotransmitter in the CNS. It has three receptor subtypes – GABA_A (postsynaptic ionotropic receptor and is a ligand-gated Cl⁻ ion channel, agonists- muscimol, isoguvacaine and antagonists- bicuculline, picrotoxin), GABA_B (presynaptic metabotropic G_i-protein coupled receptor and inhibits adenylyl cyclase, activates K⁺ channels and reduce Ca²⁺ conductance, agonist- baclofen), GABA_C (transmitter gated Cl⁻ channel). Pentameric GABA_A receptor is most abundant in the brain and has seven subunit families six α, three β, three γ and single δ, ε, π, and θ in uncertain stoichiometry. The inclusion of variant ratios of subunits in GABA_A alters the pharmacological profile of various benzodiazepines.

GABA_A receptor has various binding sites that include a GABA binding site, a modulatory site to bind benzodiazepines (at the interface between α and γ subunits, also their antagonist such as flumazenil and inverse agonist such as carbolins), the modulatory as well as blocking site at Cl⁻ ion channel as for barbiturates (α and β subunits) and picrotoxin.

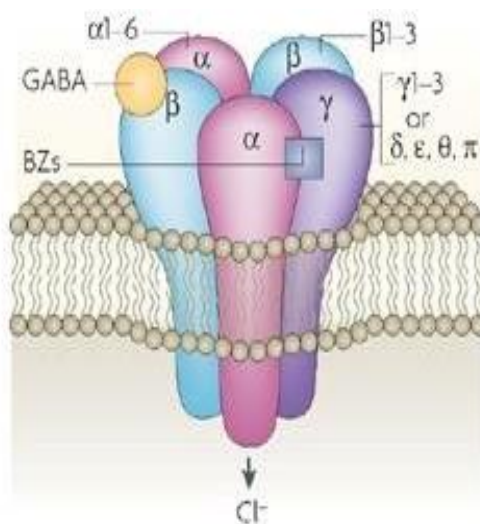


Figure 1.1 GABA_A Receptor

Benzodiazepines (BZDs) enhances GABA activity by increasing the frequency of channel opening and barbiturates potentiates GABA mediated inhibition by prolonging the duration of channel opening and at higher doses exhibits GABA mimetic action.

The most commonly found isoform of GABA_A is 2 α_1 :1 β_2 :1 γ_2 . Studies on transgenic mice led to the findings that α_1 subunits in GABA_A mediate sedation, amnesia and possibly antiepileptic actions of BZDs. α_2 subunits mediate antianxiety and muscle relaxant actions and α_5 subunit is involved in at least some of the memory impairment caused by BZDs.

Glycine and its receptors:

Glycine is another inhibitory neurotransmitter present mainly in medulla, spinal cord, lower brain stem and the retina. Its agonists are β alanine, taurine and antagonist is strychnine. Glycine receptor is also linked to Cl⁻ ion channel. Glycine and its competitive antagonist strychnine bind to α subunit while tetanus toxin acts by preventing the release of Glycine and causing excessive hyper-excitability and violent muscle spasms (lock jaw).

Glutamate, Aspartate and their receptors:

Glutamate and aspartate are the two excitatory neurotransmitters concentrated in cortex, basal ganglia and sensory pathways. Besides acting as neurotransmitters they also play a role in intermediary metabolism in neural tissue, viz., in detoxification of ammonia in brain, as building blocks in the synthesis of peptides, proteins and GABA.

There are five receptors for excitatory neurotransmitters, they are;

➤ **NMDA (N-methyl-D-aspartate) receptor:**

Ionotropic receptors linked to Ca^{2+} and involved in neurophysiological and pathological processes like memory acquisition, development of synaptic plasticity, epilepsy and neuronal excitotoxicity due to cerebral ischemia. NMDA receptor is a pentamer composed of subunits NR-1 and NR-2. It has various binding sites for binding glutamate (or NMDA), modulatory site that binds glycine (glutamate is ineffective unless glycine is bound to this site), polyamines (spermine and spermidine) binding site which facilitates channel opening, binding site for phencyclidine (PCP binding site) and related antagonists (ketamine), a voltage dependant Mg^{2+} binding site (at resting state Mg^{2+} blocks the receptor) and a voltage-independent Zn^{2+} binding site near the mouth of the channel (Zn^{2+} produces voltage independent inhibitory action on NMDA receptor ion channel).

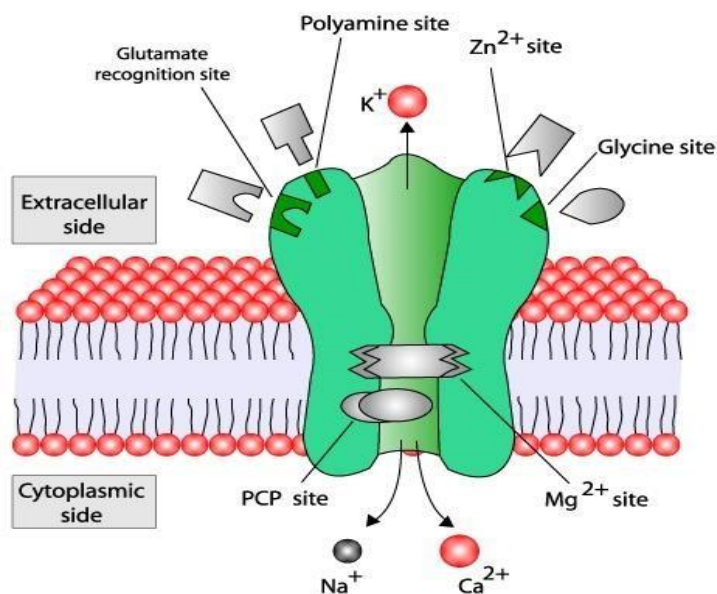


Figure 1.2. NMDA Receptor

➤ **AMPA (α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid) receptor:**

They are pentameric ionotropic receptor linked to Na^+ and consists of GLUR_{1-4} subunits. AMPA and quisqualate are the agonists.

➤ **Kainate receptor:**

They are pentameric ionotropic receptor linked to Na^+ and consists of GLUR_{5-7} and KA_{1-2} subunits. Kainate and domoate are agonists.

➤ **AP-4 (1,2-diamino-4-phosphobutyrate):**

They are inhibitory autoreceptor.

➤ **ACPD (1-aminocyclopentane-1,3-dicarboxylic acid):**

G-protein coupled receptors and either stimulate phospholipase-C- IP_3 system or inhibit and adenylyl cyclase.

Acetylcholine (ACh) and its receptors:

Cholinergic neurons are present in cerebral cortex, ascending reticular activating system, basal ganglia, limbic system, cerebellum and spinal cord. Ach modulates arousal, respiration, motor activity, vertigo and memory. Both muscarinic and N_N receptors are present in brain. Centrally acting antimuscarinic drugs are useful in Parkinsonism and anticholinesterases such as tacrine, donepezil and rivastigmine are used to improve cognitive functions in Alzheimer's disease.

1.2.2.2. Biogenic amines:

Amine neurotransmitters include dopamine, norepinephrine (epinephrine), 5-hydroxytryptamine and histamine.

Dopamine and its receptors:

Dopamine is primarily an inhibitory neurotransmitter. Its deficiency causes extrapyramidal disturbances. There are five receptors identified – D₁₋₅. Activation of D₁ and D₅ stimulate adenylyl cyclase and increase the release of cAMP; D₂₋₄ inhibits adenylyl cyclase and decrease the release of cAMP. The locations of these receptors are – D₁ – Nigrostriatal pathway (putamen, nucleus accumbens and olfactory tubercle). Its inhibition causes extrapyramidal disorders.

D₅ – Hypothalamus and hippocampus. Its exact role is not known.

D₂ – Striatum, substantia nigra and pituitary. It is involved in the control of the behavior, voluntary movements, prolactin release and other endocrine consequences.

D₃ – Midbrain, nucleus accumbens and hypothalamus.

D₄ – Mesocortical pathway (frontal cortex, medulla and midbrain). Some of the atypical neuroleptics possess D₃ and D₄ antagonistic activity.

Norepinephrine and Epinephrine:

Norepinephrine is a neurotransmitter of brainstem neurons within locus ceruleus (pons and neurons of reticular formation) with projections to cortex, cerebellum and spinal cord. In CNS it is thought to modulate affective disorders (depression), learning, memory, arousal and pain perception. Mammalian CNS contains both α - and β -adrenoceptors. Unlike dopamine and norepinephrine, concentration of epinephrine is very low and is localized primarily in reticular formation and its precise role in CNS is not known.

5-hydroxytryptamine (5-HT) and its receptors:

Serotonergic neurons are found primarily near the midline raphe nuclei of the brainstem and project to the cortex, cerebellum and the spinal cord. 90% of 5-HT is present in enterochromaffin cells and remaining 10% in brain and platelets, yet it is implicated as a potential neurotransmitter in the mediation of wide variety of brain functions. It plays important role in schizophrenia, depression, temperature regulation and eating disorders. It is a precursor of melatonin in pineal gland. It may also be involved in the hypothalamic control of the release of pituitary hormones. Its receptors are 5-HT₁₋₇ with several subtypes. All the receptors are metabotropic, except 5-HT₃ which is ionotropic. 5-HT_{1A} receptor agonists (buspirone) are used to treat anxiety disorders, 5-HT_{1D} receptor agonists (sumatriptan) are used to treat migraine and cluster headaches, 5-HT_{2A/2C} receptor antagonists (clozapine) are used to treat schizophrenia, 5-HT₃ antagonists (ondansetron) are used to prevent chemotherapy induced emesis, 5-HT₄ agonists (metoclopramide) are used as antiemetic and prokinetic drugs and 5-HT reuptake inhibitors (SSRIs- fluoxetine) are used to treat depression and obsessive-compulsive disorders.

Histamine and its receptor:

Histaminergic neurons originate from posterior hypothalamus, and project to cerebral cortex, limbic system, caudate, putamen, globus pallidus, brain stem, substantia nigra, dorsal raphe, cerebellum and spinal cord. Histaminergic neurons and postsynaptic central H₁ receptors play a major role in arousal, in coupling neuronal activity with cerebral metabolism and in neuroendocrine regulation.

Peptides:

There are number of peptide neurotransmitters in CNS involved in diverse functions of CNS besides their peripheral actions. Some of them are vasopressin (facilitate learning and memory), oxytocin (mating and parenting behavior), tachykinins (pain transmission), neurotensin (lowers body temperature), vasoactive intestinal peptide (pain transmission), endogenous opioids (analgesia, euphoria and reduces stress), cholecystokinin (appetite regulation), angiotensin II (centrally influences drinking behavior) and neuropeptide Y (increases feeding/orexigenic, hypothermia, cerebral vasoconstriction).

Imbalance among these various neurotransmitters &/or damage to the neurons or parts of CNS leads to various disorders.

1.2.3. CNS Disorders**1.2.3.1. Motor Disorders:****Epilepsy:**

Damage to the cerebral cortex and imbalance between excitatory and inhibitory neurotransmitters of brain results in epilepsy. The behavioral manifestations of a seizure are determined by the functions, normally served by the cortical site at which seizure arises.

1.2.3.2. Behavioral Disorders:

Behavioral disorders result due to damage to one or more parts of CNS, i.e., cortex, limbic system, hypothalamus and brainstem.

Depression:

Deficiency of aminergic transmission in CNS results in depression.

Mania:

Excess of aminergic transmission in CNS results in mania. It is particularly associated with changes in mood.

Schizophrenia:

Functional over activity of dopamine in limbic system or cerebral cortex leads to schizophrenia. It is particularly associated with changes in thought processes.

1.2.3.3. Neurodegenerative Disorders:**Alzheimer's disease:**

Loss of hippocampal and cortical neurons which leads to abnormalities of memory and cognitive ability.

Parkinson's disease and Huntington's disease:

Loss of neurons of basal ganglia leads to abnormalities in control of movement.

Amyotrophic lateral sclerosis (ALS):

Degeneration of spinal bulbar and cortical motor neurons lead to muscular weakness.

There are several other CNS diseases that result due to cerebrovascular accidents, physical injury to CNS, infections, etc.

1.3. INTRODUCTION TO EPILEPSY

Epilepsy is a common and frequently devastating disorder affecting millions of people worldwide. The term *seizure* refers to a transient alteration of behavior due to the disordered, synchronous and rhythmic firing of populations of brain neurons. The term *epilepsy* refers to a disorder of brain function characterized by the periodic and unpredictable occurrence of seizures. The word *fit* is often used colloquially to describe an epileptic seizure. *Convulsions* are involuntary, violent and spasmodic or prolonged contractions of skeletal muscle. That means, a patient may have epilepsy without convulsions and vice versa.

1.3.1. Signs and Symptoms of Seizures

Seizures have a beginning, middle and end. Sensory, emotional and physical symptoms appear before, during and after seizures.

➤ **Sensory / Thought Symptoms:**

Aura / early seizure symptoms (warnings)- visual loss or blurring, racing thoughts, stomach feelings, strange feelings and tingling feeling.

Seizure symptoms - Black out, confusion, deafness/sounds, electric shock feeling, loss of consciousness, smell, spacing out, out of body experience and visual loss or blurring.

Post-ictal/after-seizure symptoms - Memory loss and writing difficulty.

➤ **Emotional Symptoms:**

Aura / early seizure symptoms (warnings) - Fear/panic, pleasant feeling.

Seizure symptoms – Fear/panic.

Post-ictal/after-seizure symptoms- Confusion, depression and sadness, fear, frustration, shame/embarrassment.

➤ **Physical Symptoms:**

Aura/early seizure symptoms (warnings) - Dizziness, headache, light headedness, nausea and numbness.

Seizure symptoms - Chewing movements, convulsions, difficulty in talking, drooling, eyelid fluttering, eyes rolling up, falling down, foot stomping, hand waving, inability to move, incontinence, lip smacking, making sounds, shaking, staring, stiffening, swallowing, sweating, teeth clenching/grinding, tongue biting, tremors, twitching movements, breathing difficulty and heart racing.

Post-ictal/after-seizure symptoms - Bruising, difficulty talking, injuries, sleeping, exhaustion, headache, nausea, pain, thirst, weakness, urge to urinate/defecate.

1.3.2. Age and Onset of Epilepsy

Epilepsy primarily affects the very young and the very old, although anyone can get epilepsy at anytime. Twenty percent of cases develop before the age of five. Fifty percent develop before the age of 25.

1.3.3. Cause of Epilepsy

In about 70 percent of cases there is no known cause. Of the remaining 30 percent, the following are the most frequent causes:

- Arteriovenous malformation (AVM)
- Head injury
- Intoxication with drugs
- Drug toxicity, eg. aminophylline or local anesthetics
- Brain tumors
- Normal doses of certain drugs that lower the seizure threshold, eg. Tricyclic antidepressants
- Infections, eg. encephalitis or meningitis
- Febrile convulsions
- Metabolic disturbances, eg. hypoglycemia, hyponatremia or hypoxia
- Sudden withdrawal of certain drugs eg. Anticonvulsants and sedatives such as barbiturates, and benzodiazepines; alcohol.
- Space-occupying lesions in the brain (abscesses, tumors)
- Eclampsia.
- Binaural beat brainwave entrainment may trigger seizures in both epileptics and non-epileptics
- Stroke may cause seizures, eg. embolic strokes, cerebral venous sinus thrombosis.

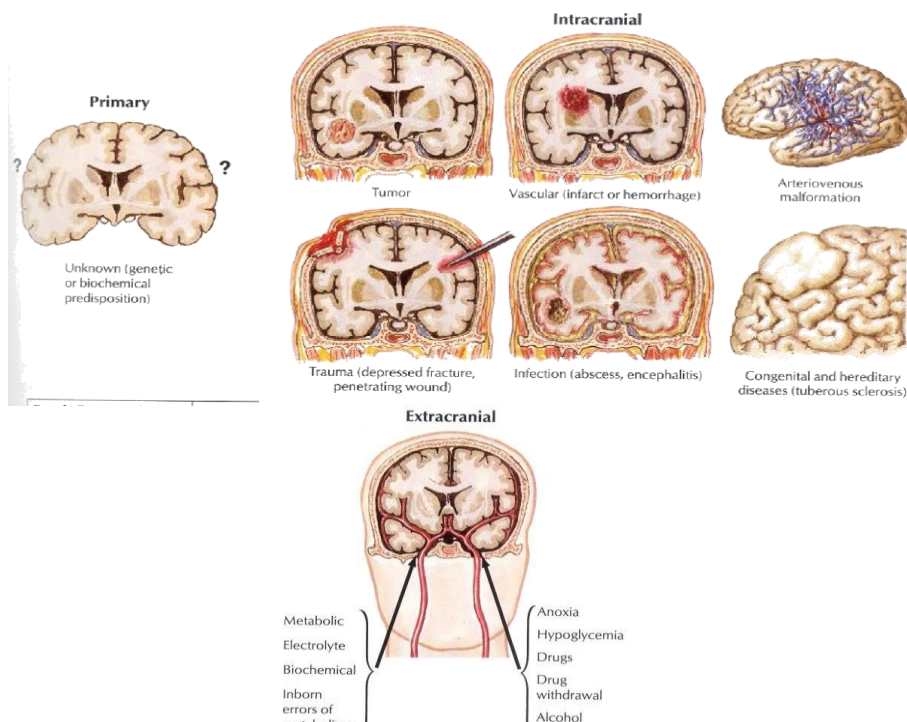


Figure 1.3. Causes of Seizures

1.3.4. Classification of Seizures

1.3.4.1. Partial Seizures (Localized/Focal Seizures):

➤ Simple Partial Seizures (Jacksonian Epilepsy) (SPS):

Duration 20-60sec, consciousness is not impaired. SPS results from rapid neuronal discharges in one part of the brain, usually the cortex or limbic system. These seizures take different forms like motor, sensory, autonomic and psychic and are characterized by strange or unusual sensations, for example odors or visual abnormalities, sudden or restless movement, hearing distortion, stomach discomfort, and a sudden sense of fear. The International League Against Epilepsy (ILAE) introduced International Classification for Epilepsies and Epileptic Syndromes (ICES) which categorized simple partial seizures into four subtypes.

- **Motor:** (cause changes in muscle activity. For example, a person may have abnormal movements such as jerking of a finger or stiffening of part of the body).
- **Sensory:** (cause changes in any one of the senses. People with sensory seizures may smell or taste things that aren't there; hear clicking, ringing, or a person's voice when there is no actual sound; or feel a sensation of "pins and needles" or numbness).
- **Psychic:** (cause changes in how patients think, feel, or experience things. They may have problems with memory, garbled speech, and inability to find the right word, or trouble understanding spoken or written language. They may suddenly feel emotions like fear, depression, or happiness with no outside reason. Some may feel as though they are outside their body).
- **Autonomic:** (cause changes in the part of the nervous system that automatically controls bodily functions. These include strange or unpleasant sensations in the stomach, chest, or head; changes in the heart rate or breathing; sweating; or goose bumps). Simple partial seizures lead to complex partial seizures or to tonic-clonic convulsions.

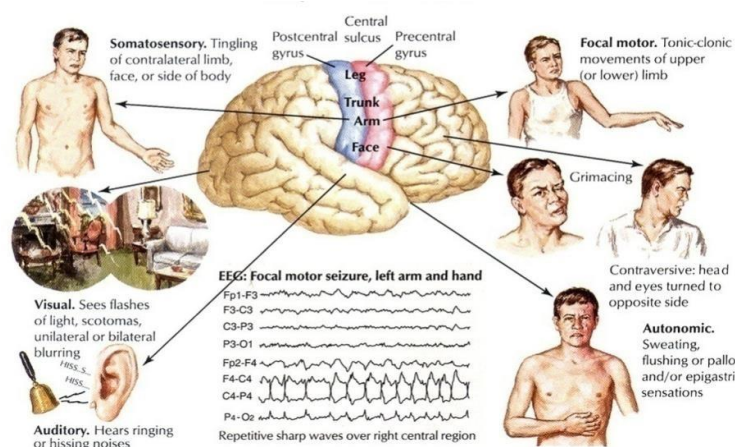


Figure 1.4. Simple Partial Seizures

➤ **Complex Partial (CPS/Psychomotor/Temporal Lobe Epilepsy) Seizures:**

Duration 30sec-2min with impaired consciousness. CPS occurs when epileptic activity spreads to involve a major portion of the brain but does not become generalized. They often are preceded by aura and occur after a simple partial seizure particularly when it is of temporal lobe origin. CPS often begin with a blank look or stare and then may progress to chewing or uncoordinated activity, meaningless bits of behavior, which appear random and clumsy (automatisms) and patients may appear afraid, try to run and struggle. These seizures are followed by a state of confusion that lasts even longer. Once the pattern of the seizures is established it will usually be repeated with each subsequent seizure. CPS sometimes resists anticonvulsant medication. In some cases CPS may lead to tonic-clonic seizures.

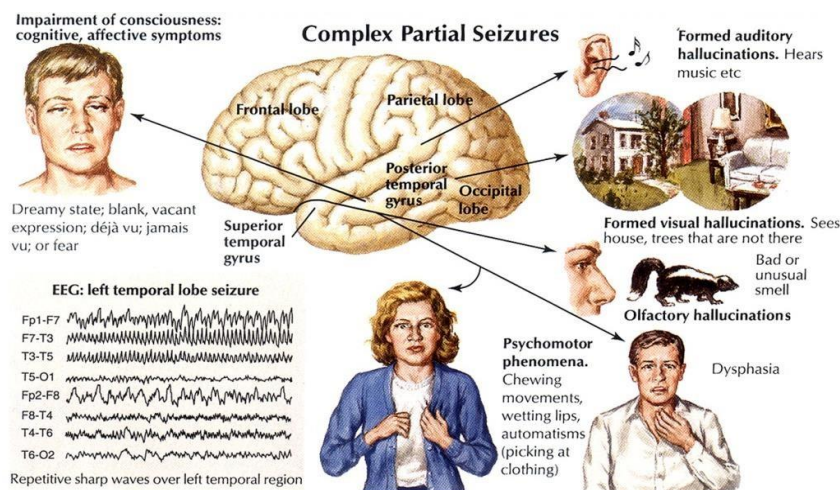


Figure 1.5. Complex Partial Seizures

➤ **Partial Seizures evolving to Secondly Generalized Seizures:**

Duration 1-2min. Seizures of this kind start as a partial seizure that is, they start in one limited area of the brain and then (sometimes so quickly that the partial seizure is hardly noticed) the seizure spreads throughout the brain, becoming "generalized."

1.3.4.2. Generalized Seizures:

➤ Absence (Petit mal) Seizures:

Epileptic activity occurs throughout the entire brain. It is a milder type of activity, however, causes unconsciousness without causing convulsions. An absence seizure consists of a period of unconsciousness with a blank stare, and begins and ends abruptly, without warning. There is no confusion after the seizure; seizures may be accompanied by chewing movements, rapid breathing, or rhythmic blinking. Absence seizures are short, usually lasting 2-10 seconds. They are very mild, and may go unnoticed and may recur frequently during the day.

Typical Absence Seizures:

They are non-convulsive and muscle tone is usually preserved. The seizure event usually lasts for less than 10 seconds in duration.

Atypical Absence Seizures:

They are longer in duration than typical absence seizures with or without loss in muscle tone and often tonic/clonic-like movements are observed.

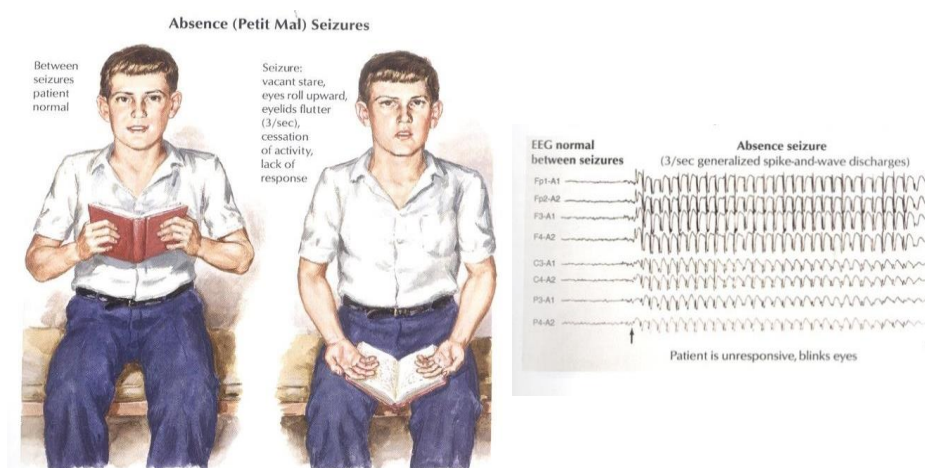


Figure 1.6. Absence Seizures

➤ **Myoclonic Seizures:**

Occur in several different types of epilepsy. The seizures involve abrupt muscle jerks in part or all of the body, eg. a hand suddenly flinging out, shoulder shrug, foot kicking, or the whole body may jerk. The events may occur individually, or in a series. Consciousness is not impaired. Myoclonic Seizures are not tics or “startle” responses.

➤ **Clonic seizures:**

They are characterized by repetitive muscle jerks.

➤ **Tonic Seizures:**

Characterized by rigid violent muscular contraction with stiff and fixed extended limbs.

➤ **Tonic-Clonic (Grand mal) Seizures:**

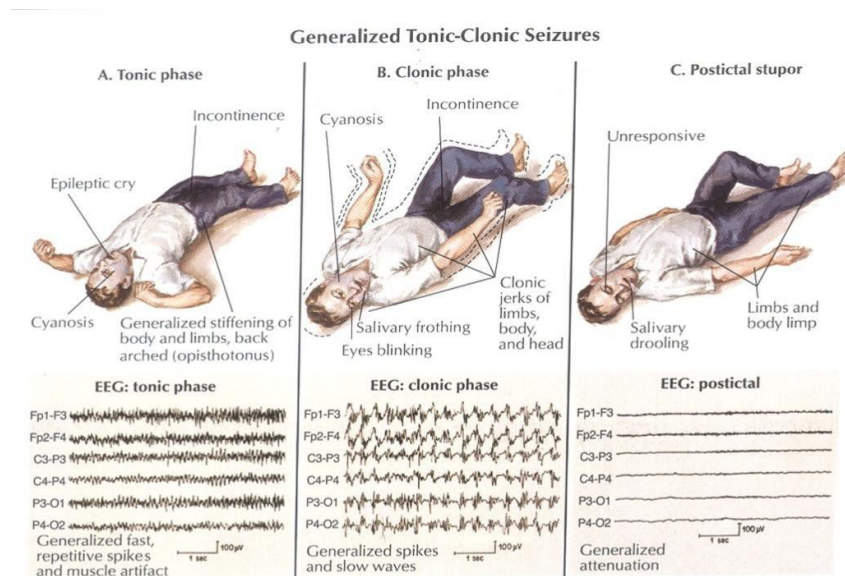


Figure 1.7. Generalized Tonic-Clonic Seizures

Generalized seizures occur when epileptic activity occurs throughout the brain. Patient becomes unconscious from the start, and will have a major convulsion with both tonic (stiffening) and clonic (jerking) phase. After the seizure, the patients are unconscious and then groggy for a while. They may want to sleep. There will be no memory of what went on during the seizure. The seizure begins with a fall, possibly accompanied by a sudden cry, followed by tonus and then, after a while, clonus. There may be shallow breathing or temporarily suspended breathing, with bluish skin or lips and loss of bladder or bowel control. Towards the end of the seizure, patient may salivate profusely. Tonic-clonic seizures usually last 1 to 3 minutes, seldom longer.

➤ **Atonic Seizures:**

Although relatively uncommon, they are hard to deal with as they occur without warning. The individual abruptly loses consciousness, collapses and falls to the floor. There is no convulsion, but the patients may injure themselves as they fall. Recovery occurs after a few seconds.

1.3.4.3. Unclassified Epileptic Seizures (Status Epilepticus):

Status epilepticus (SE) refers to continuous seizure activity with no recovery between successive seizures. It is a life-threatening condition. A tonic-clonic seizure lasting longer than 5 minutes (or two minutes longer than a given person's usual seizures) is usually considered grounds for calling the emergency services.

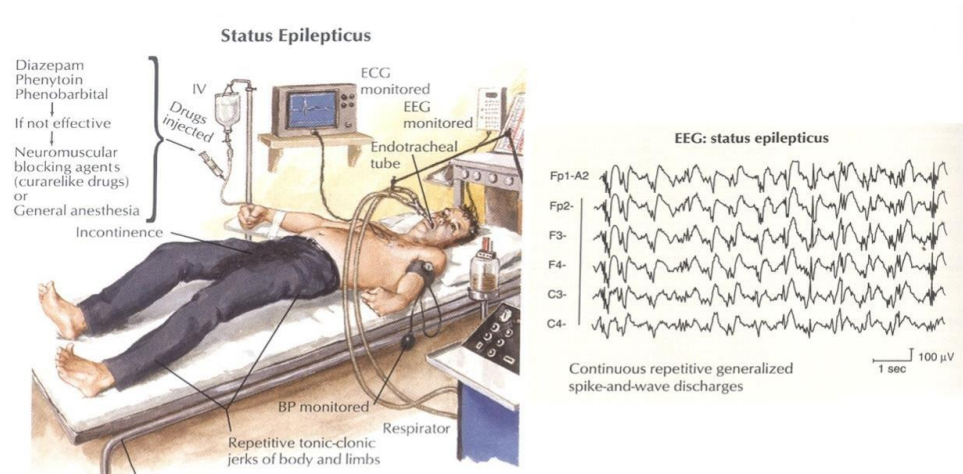


Figure 1.8. Status Epilepticus

1.3.5. Seizure Syndromes:

There are many different epilepsy syndromes, each presenting with its own unique combination of seizure type, typical age of onset, EEG findings, treatment, and prognosis. Below are some common seizure syndromes:

- Infantile Spasms (West syndrome)
- Childhood Absence Epilepsy
- Dravet's Syndrome
- Benign Focal Epilepsies of Childhood
- Juvenile Myoclonic Epilepsy (JME)
- Temporal Lobe Epilepsy
- Fetal Alcohol Syndrome
- Frontal Lobe Epilepsy
- Lennox-Gastaut Syndrome

1.3.6. Endogenous Anti-seizure Substances

It is suggested that some sort of regulatory mechanism must be existing in the body as spontaneous arrest of seizure activity occurs after an attack and also brain remains seizure free for sometime between the two intervening attacks (postictal refractory period). Elevated adenosine levels have been reported immediately after the seizure activity both in animal models as well as in patients. Adenosine has been shown to inhibit spontaneous firing of cells in virtually all areas of brain including cerebral cortex. It causes hyperpolarization and exhibits A₁ receptor mediated anticonvulsant effects in animal models when administered exogenously. Since it is released postictal and it is not tonically active, A₁ receptor antagonists per se do not exhibit any convulsant activity (www.shodhganga.inflibnet.ac.in)

1.4. INTRODUCTION TO ANXIETY

Anxiety is a subjective feeling of unease, discomfort, apprehension or fearful concern accompanied by a host of autonomic and somatic manifestations. Anxiety is a normal, emotional, reasonable and expected response to real or potential danger. However, if the symptoms of anxiety are prolonged, irrational, disproportionate and/or severe; occur in the absence of stressful events or stimuli; or interfere with everyday activities, then, these are called Anxiety Disorders (DSM IV-TR, 2000). Anxiety disorders are among the most common mental, emotional, and behavioral problems (Kessler *et al.*, 2005a, 2005b; Olatunji *et al.*, 2007; Kessler & Wang, 2008). These affect one-eighth of the total population worldwide, and have become a very important area of research interest in psychopharmacology (Eisenberg *et al.*, 1998; Dopheide & Park, 2002; WHO, 2004).

In addition to the high prevalence, anxiety disorders account for major expenditure for their management (DuPont *et al.*, 1996); and anxiety disorders have a substantial negative impact on quality of life (Gladis *et al.*, 1999; Mendlowicz & Stein, 2000; Olatunji *et al.*, 2007).

1.4.1. Symptoms of Anxiety Disorders:

The subjective experience of anxiety typically has two components namely physical component and emotional component which affect the cognitive processes of the individual (Cates *et al.*, 1996; Charles and Shelton, 2004; Augustin, 2005; Shri, 2006; Rang *et al.*, 2007) and these have been shown in F

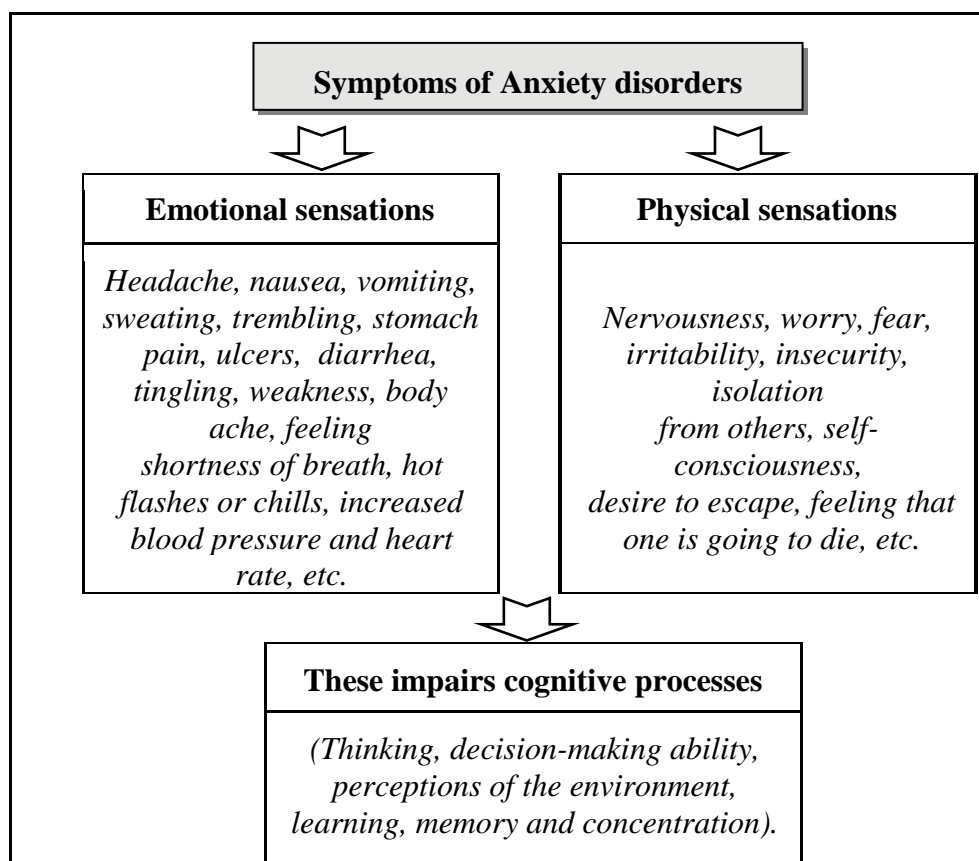


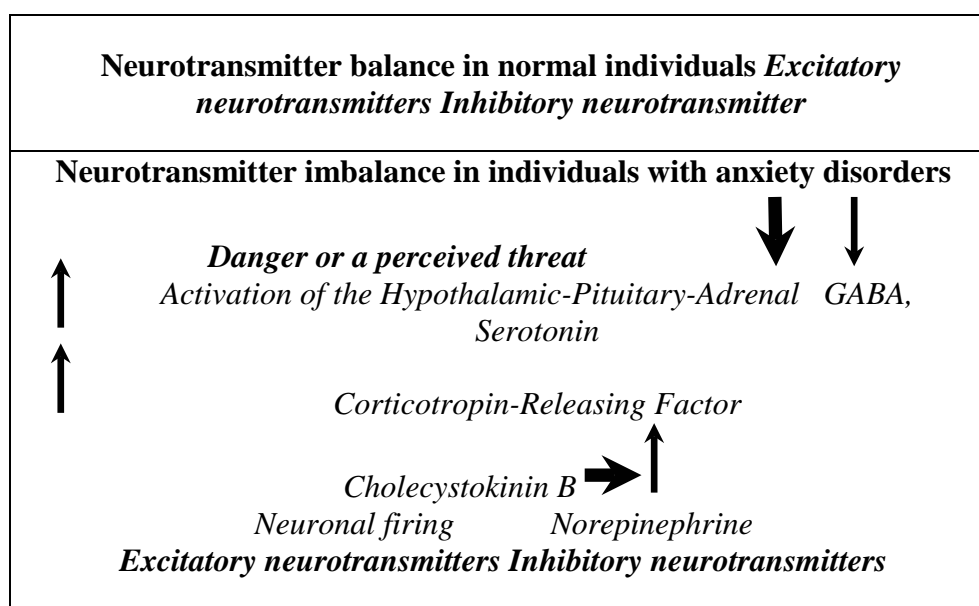
Figure 1.9. Symptoms of anxiety

Table. 1.1. Etiology of Anxiety Disorder

| Biological causes | Psychological causes | Social causes |
|--|---|--|
| Hereditiy Neurotransmitter imbalance Illness Medications Nutritional factors | Personality traits Low self-esteem Cognitive dissonance Negative emotions Inter and/or intra-personal conflicts | Adverse Life Experiences Lack of social support Work stress Lack of social skills Changing values Conflict of societal norms Terrorism |

1.4.2. Etiology:

Anxiety disorders are among the most frequent mental disorders encountered in clinical practice (Kirkwood & Melton, 2002). These represent a heterogenous group of disorders, probably with no single unifying etiology. Various psychodynamic, psychoanalytic, behavioral, cognitive, genetic and biological theories have been proposed to explain the etiology and pathophysiology of anxiety disorders (Cates *et al.*, 1996). These are said to be Bio-Psycho-Social factors that contribute to anxiety disorders (Pies, 1994; White, 2005; Wong, 2006).

**Figure 1.10. Neurotransmitters involved in occurrence of anxiety disorders.**

1.4.2.1. Biological factors

➤ **Genetic factors:**

Genetic factors predispose certain people to anxiety disorders. There is a higher chance of an anxiety disorder in the parents, children and siblings of a person with an anxiety disorder than in the relatives of someone without an anxiety disorder (Torgersen, 1983; Weissman, 1993; Goldman, 2001).

➤ **Neurotransmitter imbalance:**

Brain imaging and functional studies have shown that several neurotransmitters are linked to the neurobiology of anxiety (Cates *et al.*, 1996 Sandford *et al.*, 2000; Millan, 2003; Augustin, 2005).

1.4.2.2. Psychological factors:

Anxiety can result when a combination of increased internal and external stresses overwhelm one's normal coping abilities or when one's ability to cope normally is lessened for some reason. The psychological factors are summarized below:

- **Psychodynamic:** When internal competing mental processes, instincts and impulses conflict, causing distress.
- **Behavioral:** Anxiety is a maladaptive learned response to specific past experiences and situations that become generalized to future similar situations.
- **Spiritual:** When people experience a profound, unquenchable emptiness and nothingness to their lives, often leading to distress concerning their mortality and eventual death (Sarason & Sarason, 2000; Brannon & Feist, 2004).

1.4.2.3. Social factors

Life experiences like death in the family, divorce, job loss, financial loss, accident or major illness affect a person's attitude and response to life situations. Long term exposure to abuse, violence, terrorism and poverty may affect an individual's susceptibility to anxiety disorders (Eysenck, 2004).

1.4.3. Types of Anxiety Disorders:

Anxiety disorders can be classified into several categories (ICD-10, 1992; Cates et al., 1996; DSM-IV-TR, 2000; Augustin, 2005; Rang et al., 2007). As shown in table 2 the different types of anxiety disorders and their clinical symptoms can be differentiated.

Table.1.2. Type of Anxiety Disorders and Their Symptoms

| Anxiety disorder | Clinical Symptoms |
|---|--|
| Generalized anxiety disorder | Excessive and unrealistic worry that is difficult to control about several life circumstances for 6 months or longer |
| Panic disorder (With/without agoraphobia) | Occurrence of recurrent, unexpected attacks of overwhelming fear occurring in association with marked somatic symptoms, such as sweating, tachycardia, chest pains, trembling, choking, etc. |
| Agoraphobia without history of panic disorder | Irrational and often disabling fear of public places or open areas |
| Phobic disorders Social phobia Specific phobias | Strong fears of specific things or situations, e.g., snake, open spaces, flying, social interactions, etc. |
| Post-traumatic stress disorder | Anxiety triggered by insistent recall of past stressful/traumatic experiences |
| Separation anxiety disorder | Difficulty in leaving dear ones |
| Obsessive-compulsive disorder | When one is trapped in a pattern of repetitive thoughts and behaviors, i.e. recurrent obsessions or compulsions that cause marked distress; are time consuming; or interfere significantly with normal occupational functioning, social activities, or relationships |

| | |
|---|--|
| Acute stress disorder | Anxiety reaction which may occur shortly after traumatic exposure |
| Anxiety disorder due to a general medical condition | Knowledge that one has chronic and perhaps disabling medical illness can precipitate anxiety |
| Substance induced anxiety disorder | Anxiety related to substance abuse |
| Anxiety disorder not otherwise specified | Anxiety reactions which do not fall in any of above categories |

1.4.4. Management of Anxiety

Anxiety disorders are the most prevalent of psychiatric disorders, yet less than 30% of individuals who suffer from anxiety disorders seek treatment (Lepine, 2002). People with anxiety disorders can benefit from a variety of treatments and services. Following an accurate diagnosis, possible treatments include (Barlow, 2001; NIMH, 2006) psychological treatments and medication.

1.4.4.1. Psychological Therapies:

Psychotherapy is almost always the *treatment of choice* except in cases where anxiety is so severe that immediate relief is necessary to restore functioning and to prevent immediate and severe consequences. This includes the following.

Behavioral therapy:

These focus on using techniques such as guided imagery, relaxation training, biofeedback (to control stress and muscle tension); progressive desensitization, flooding as means to reduce anxiety responses or eliminate specific phobias. The person is gradually exposed to the object or situation that is feared. At first, the exposure may be only through pictures or audiotapes. Later, if possible, the person

actually confronts the feared object or situation. Often the therapist will accompany him or her to provide support and guidance.

➤ **Cognitive-behavioral therapy (CBT):**

In this therapy, people learn to deal with fears by modifying the ways they think and behave. A major aim of CBT and behavioral therapy is to reduce anxiety by eliminating beliefs or behaviors that help to maintain the anxiety disorder. Research has shown that CBT is effective for several anxiety disorders, particularly panic disorder and social phobia (Herbert et al., 2009). It has *two* components. The *cognitive component* helps people change thinking patterns that keep them from overcoming their fears. The *behavioral component* of CBT seeks to change people's reactions to anxiety-provoking situations. A key element of this component is exposure, in which people confront the things they fear, i.e., CBT addresses underlying “automatic” thoughts and feelings that result from fear, as well as specific techniques to reduce or replace maladaptive behavior patterns.

➤ **Psychotherapy:**

Psychotherapy centers on resolution of conflicts and stresses, as well as the developmental aspects of anxiety disorders solely through talk therapy. Psychotherapy involves talking with a trained mental health professional, such as a psychiatrist, psychologist, social worker, or counselor to learn how to deal with problems like anxiety disorders (Knekt *et al.*, 2008).

➤ **Psychodynamic therapy:**

This therapy, first suggested by Freud, is based on the premise that primary sources of abnormal behavior are unresolved past conflicts and the possibility that unacceptable unconscious impulses will enter consciousness.

➤ **Family therapy and parent training:**

Here the focus is on the family and its dynamics. This is based on the assumption that the individuals of a family cannot improve without understanding the conflicts that are to be found in the interactions of the family members. Thus, each member is expected to contribute to the resolution of the problem being addressed (American Psychological Association, 2004; Feldman, 2004).

Table 1.3 : Major Classes of Medications Used for Various Anxiety Disorders

| Class | Generic name | Used for | Mechanism of action | Advantages | Limitations |
|------------------------|---|--------------------------------|--------------------------------------|--|---|
| Anticonvulsants | Gabapentin | SAD | Affect GABA | Usually effective within 2-4 weeks | Sedation |
| Azaspirones | Buspirone | GAD | Enhances the activity of serotonin | Less sedating than benzodiazepines | Works slowly |
| Benzodiazepines | Lorazepam Clonazepam Oxazepam Diazepam Alprazolam | GAD, SAD, Panic disorder | Enhance the function of GABA | Fast-acting, some people feel better the first day | Potentially habit-forming, can cause drowsiness, can produce withdrawal symptoms, discontinuation should be done slowly |
| Beta blockers | Propranolol Atenolol | SAD | Reduce ability to produce adrenaline | Fast acting; not habitforming | Should not be used with pre-existing medical conditions, such as asthma, congestive heart failure, diabetes, vascular disease, hypothyroidism and angina pectoris |

| | | | | | |
|--|---|-------------------------------|--|--|---|
| Monoamine oxidase inhibitors (MAOIs) | Selegilene Isocarboxid Phenelzine Tranlycypromine | Panic disorder, SAD, PTSD | Block the effect of an important brain enzyme, preventing the breakdown of serotonin and noradrenaline | Effective for many people, especially for patients not responding to other medications, 2-6 weeks until improvement occurs | Strict dietary restrictions and potential drug interactions, changes in blood pressure, moderate weight gain, reduced sexual response, insomnia |
| Selective serotonin reuptake inhibitors (SSRIs) | Citalopram Fluvoxamine Paroxetine Fluoxetine Sertraline | Panic disorder, OCD, SAD, GAD | Affect the concentration of serotonin | Effective, with fewer side effects than other medications. 4-6 weeks until improvement occurs | Some people experience nausea, nervousness and diminished sex drive |
| Tricyclic antidepressants (TCAs) | Nortriptyline Amitriptyline Imipramine | Panic disorder, PTSD, OCD | Regulates serotonin and/or noradrenaline in the brain | Effective for many people, may take 2-6 weeks until improvement occurs. | Dry mouth, constipation, blurry vision, difficulty urinating, dizziness, low blood pressure, moderate weight gain, sexual side effects |

Note. GAD = Generalized anxiety disorder, OCD = Obsessive compulsive disorder,

PTSD = Post Traumatic stress disorder, SAD=Social anxiety

1.4.5. Plants used for management of anxiety

The World Health Organisation estimates that 80% of the world population relies on herbal medicine (Eisenberg *et al.*, 1998). Various plants have been investigated for their anxiolytic effects (Carlini, 2003) and many have shown marked antianxiety activity. Monoherbal preparations containing *Scutellaria laterifolia*, *Centella asiatica*, *Paullinia cupana*, *Piper methysticum*, *Bacopa monniera*, *Cymbopogon citratus*, *Passiflofa incarnata* and *Valeriana officinalis* were subjected to randomised clinical trials to study their effect in alleviation of anxiety (Ernst, 2006). According to the reported data, *Piper methysticum* (Pittler *et al.*, 2002) and *Bacopa monniera*, (Stough *et al.*, 2001) are associated with anxiolytic activity in humans. In another trial on generalized anxiety disorder (GAD) in hospital based clinical set-up, *Ocimumn sanctum* significantly attenuated generalized anxiety disorders and also attenuated its correlated stress and depression (Bhattacharyya *et al.*, 2008).

CHAPTER 2

LITERATURE REVIEW

Literature review is the first and most important step for the proper selection of plants and it also forms basis for the planning of any scientific work that has to be performed. Due to this reason, the review of literature regarding *Syzygium aqueum* has been done under various divisions like Pharmacognostical, Phytochemical, Pharmacological, Ethno medical and also miscellaneous reviews.

- Azza A. A. Galal *et al.*, has reported **Neuropharmacological studies on *Syzygium aromaticum* essential oils** on International Journal of Pharma science ISSN-2320-6810, Vol.5, No.2 (2015); 1013-1018.
- Shabnam Millika *et al.*, has reported **Analgesic, Anti-inflammatory and CNS activities of Methanolic extract of *Syzygium samarangense* bark** on IOSR Journal of pharmacy (e) ISSN:2250-3013, (p) ISSN:2319-4219, www.iosrphr.org, vol.3, Dec 2013, pp 12-18.
- Patali Snehalatha. N *et al.*, has reported **Antiepileptic activity of Methanolic extract of *Syzygium cumini* seeds in Albino mice** on International Journal of Pharmacy and Technology, ISSN:0975-766X, vol.5, Issue No.3, Nov 2013, 5697-5704.
- Shabnam Millika *et al.*, has reported **Analgesic, Anti-inflammatory and CNS activities of Methanolic extract of *Syzygium samarangene* leaves** on Global Journal of Pharmacology 8(1):39-46, 2014, ISSN:1992-0075.

- Manaharan. T *et al.*, has reported ***Syzygium aqueum* leaf extract and it's Bio active compounds enhance pre-adipocyte differentiation and 2-NBDG uptake in 3T3-L1 cells** on Research gate, Food chemistry 136 (2013), 354-363.
- Levitah.C *et al.*, has reported **Efficacy of Three varieties of *Syzygium aqueum* (Tambis) as Antimicrobial agent and it's Bio active component** on International Journal of Science and Clinical laboratory, vol.5, No.1 (2014).
- Rabeta *et al.*, has reported **Anticancer effect of underutilized fruits (*Syzygium aqueum*, *Syzygium malaccense*, *Syzygium malaccense L.*)** on International Food Research journal 20 (2), 2013; 551-556.
- Manaharan.T *et al.*, has reported **Flavanoids isolated from *Syzygium aqueum* leaf extract as potential Anti-hyperglycaemic agents** on Research gate, Food chemistry 132 (2012), 1802-1807.
- Monemzzaman khandaker. M *et al.*, has reported **Bio active constituents, Antioxidant and Antimicrobial Activities of Three cultivators of Wax apple Fruits** on Research Journal of Biotechnology, vol.10, January 2015.
- Nallakurumban. B *et al.*, has reported **Phytochemical and Antioxidant Properties of Water apple (*Syzygium aqueum*) and Chapathikalli (*Optunia ficus indica*)** on International Journal for Research in Emerging Science and Technology, vol.2, Issue 10, Oct 2015, E.ISSN 2349-7610.

CHAPTER 3

AIM AND PLAN OF WORK

3.1 AIM OF PRESENT STUDY

In recent year there has been a tremendous increase in demand for herbal drugs due to its safety, efficacy and better therapeutic results and also due to its economic pricing as compared to synthetic or allopathic drugs, which have several therapeutic complications.

The selection of this plant, *Syzygium aqueum* was made on the basis of its

- ✓ High therapeutic value
- ✓ Easy availability
- ✓ Degree of research work which is not done

Very less pharmacological studies have been carried out on the leaves of *Syzygium aqueum*. Hence, I have decided to choose *Syzygium aqueum* on which detailed studies on Preliminary Phytochemical and Pharmacological actions on CNS is done.

3.2 THE PLAN OF WORK

The plan of work for the study of *Syzygium aqueum* was carried out as follow.

Collection and authentication of raw material

1. Preliminary phytochemical studies
 - a. Preparation of extract
 - b. Qualitative phytochemical studies
2. Pharmacological studies
 - a. Acute oral Toxicity study
 - b. Screening of Anxiolytic activity
 - c. Screening of Anticonvulsant activity

CHAPTER 4

PLANT PROFILE

Plant Classification:

| | | |
|-------------|---|--|
| Kingdom | : | Plantae |
| Order | : | Myrtales |
| Family | : | Myrtaceae |
| Genus | : | Syzygium |
| Species | : | S.aqueum |
| Synonym | : | Eugenia aquea |
| Local names | : | Watery rose apple, Water apple, Bell fruit |



Fig. 3.1. Tree of *Syzygium aqueum*



Fig. 3.2. Leaves of Syzygium aqueum



Fig. 3.3. Fruits of Syzygium aqueum

Plant Description:

The *Syzygium aqueum* (Brum.f) Alston is an evergreen, 3-10 m tall tree, ramified until the base, with brown bark, with which the age gets fissured and peels off, and thick and irregular foliage.

The leaves on short petiole, are opposite, obovate or elliptic-oblong, cordate at the base, 5-22 cm long and 3-10 cm broad, of pale green color on the upper page, yellowish green below, leathery.

The inflorescences are axillar and terminal, on a short peduncle, carrying 3-7 flowers with 4 spatulate petals, about 0.7 cm long, of pale yellow or pinkish color and several stamina, of the same color of petals, up to 2 cm long.

The fruits are piriform or bell shaped berries, flattened at the two ends, 1.5-2 cm long and 2.5-3.5 cm broad, of pink or red color with white or pinkish pulp, crunchy or spongy, aqueous, refreshing with a light sweetish taste. The fruits may be seedless or may contain 1-6 seeds, most frequently 1-2 (www.photomazza.com).

CHAPTER 5

MATERIALS AND METHODS

5.1 COLLECTION AND IDENTIFICATION

5.1.1 Collection of specimen:

The species for the proposed study that is leaves of *Syzygium aqueum* has carefully collected from Angadippuram, Malappuram DT, Kerala.

5.1.2 Taxonomical identification:

The plant was positively identified by Dr. Prabhukumar.K.M, Senior Scientist and Head, Plant Systematics and Genetic Resource Division and CMPR Herbaria, Centre for Medicinal Plant Research, Arya Vaidya Sala, Kottakkal. The plant was authenticated as *Syzygium aqueum* (Brum.f.) Alston of Myrtaceae family.

5.1.3 Shade drying:

After collection, the leaves of *Syzygium aqueum* were washed thoroughly with water to remove the dirt particles and any other foreign material adheres to leaves. Then after, the leaves were wiped off with cotton cloth and transferred to newspaper and evenly spreader on to paper.

The *Syzygium aqueum* leaves were subjected to shade drying to treat fungus until complete dryness of leaves. Then the dried leaves were powdered by mixer grinder until to get coarse powder, which was used for further detailed studies, extraction with solvent and phytochemical studies.

5.2. PRELIMINARY PHYTOCHEMICAL ANALYSIS

Extraction of *Syzygium aqueum* leaves:

Methanol extract:

About 250gm of air dried powdered material was taken in 3000ml soxhlet apparatus and extracted with petroleum ether until green colour disappear. At the end of the day the powder was taken out and dried. After drying it was again packed and extracted by using Methanol (S.D. Fine Chemicals Ltd. Mumbai, India) as solvent, till colour disappeared. The temperature was maintained at 55°C-65°C. After that extract was concentrated by distillation and solvent was recovered. The final solution was evaporated to dryness. The colour, consistency and yield of Methanolic extract were noted.

Table: 5.1 Nature of extract of *Syzygium aqueum*

| S.No. | Name of extract | Colour | Consistency | Yield% W/W |
|-------|--------------------|----------------|-------------|------------|
| 1. | Methanolic extract | Greenish black | Sticky mass | 7 |

5.3 CHEMICAL TESTS:

A) Test for carbohydrates:

1. Molisch's Test: It consists of treating the compounds of α -naphthol and concentrated sulphuric acid along the sides of the test tube.

Purple colour or reddish violet colour was produced at the junction between two liquids. (Kokate, C.K *et al.*, 2000)

2. Fehling's Test: Equal quantity of Fehling's solution A and B is added. Heat gently, brick red precipitate is obtained.

3. Benedict's test: To the 5ml of Benedict's reagent, add 8 drops of solution under examination. Mix well, boiling the mixture vigorously for two minutes and then cool. Red precipitate is obtained

B) Test for Alkaloids:

1. Dragendroff's Test: To the extract, add 1ml of Dragendroff's reagent, Orange red precipitate is produced.

2. Wagner's test: To the extract add Wagner reagent, reddish brown precipitate is produced.

3. Mayer's Test: To the extract add 1ml or 2ml of Mayer's reagent, dull white precipitate is produced.

4. Hager's Test: To the extract add 3ml of Hager's reagent, yellow Precipitate is produced.

C) Test for Steroids and Sterols:

1. Liebermann Burchard test: Dissolve the test sample in 2ml of chloroform in a dry test tube. Now add 10 drops of acetic anhydride and 2 drops of concentrated sulphuric acid. The solution becomes red, then blue and finally bluish green in colour.

2. **Salkowski test:** Dissolve the sample of test solution in chloroform and add equal volume of conc. sulphuric acid. Bluish red cherry red and purple color is noted in chloroform layer, whereas acid assumes marked green fluorescence.

D) Test for Glycosides:

1. **Legal's test:** Sample is dissolved in pyridine; sodium nitropruside solution is added to it and made alkaline. Pink red colour is produced.
2. **Baljet test:** To the drug sample, sodium picrate solution is added. Yellow to orange colour is produced.
3. **Borntreger test:** Add a few ml of dilute sulphuric acid to the test solution. Boil, filter and extract the filtrate with ether or chloroform. Then organic layer is separated to which ammonia is added, pink, red or violet colour is produced in organic layer.
4. **Killer Killani test:** Sample is dissolved in acetic acid containing trace of ferric chloride and transferred to the surface of concentrated sulphuric acid. At the junction of liquid reddish brown color is produced which gradually becomes blue.

E) Test for Saponins:

Foam test: About 1ml of alcoholic sample is diluted separately with distilled water to 20ml, and shaken in graduated cylinder for 15 minutes. 1 cm layer of foam indicates the presence of saponins.

F) Test for Flavonoids:

Shinoda test: Red colour is produced when the sample, magnesium turnings and then concentrated hydrochloric acid is added.

Ferric chloride test – Test solution when treated with few drops of Ferric chloride solution would result in the formation of blackish red color indicating the presence of flavonoids.

Alkaline reagent Test – Test solution when treated with sodium hydroxide solution, shows increase in the intensity of yellow color which would become colorless on addition of few drops of dilute Hydrochloric acid, indicates the presence of flavonoids.

Lead acetate solution Test – Test solution when treated with few drops of lead acetate (10%) solution would result in the formation of yellow precipitate.

G) Test for Tri-Terpenoids:

In the test tube, 2 or 3 granules of tin was added, and dissolved in a 2ml of thionyl chloride solution and test solution is added. Pink colour is produced which indicates the presence of triterpenoids.

H) Tests for Tannins and Phenolic Compounds:

The Phenol content in the raw material of caesalpiniasappanextract was estimated by spectroscopically method.

To 2-3 ml of extract, add few drops of following reagents:

- a) **5% FeCl₃ solution:** deep blue-black color.
- b) **Lead acetate solution:** white precipitate.
- c) **Gelatin solution:** white precipitate
- d) **Bromine water:**decolouration of bromine water.
- e) **Acetic acid solution:** red color solution
- f) **Dilute iodine solution:** transient red color.
- g) **Dilute HNO₃:** reddish to yellow color

5.4. PHARMACOLOGICAL EVALUATION

5.4.1. ACUTE ORAL TOXICITY STUDY

The procedure was followed by using OECD guidelines 423 (Acute toxic class method).The acute toxic class method is a step wise procedure with 3 animals of single sex per step. Depending on the mortality and / or moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test animals while allowing for acceptable data based scientific conclusion.

The method uses defined doses (5, 50, 300, 2000mg/kg body weight) and the results allow a substance to be ranked and classified according to the Globally Harmonized System (GHS) for the classification of chemical which cause acute toxicity.

ANIMALS:

Female albino mice of 20-30 gm of body weight obtain from Animal House, Department of Pharmacology, J.K.K.M.M.R.F. College of pharmacy, Namakkal DT, Tamil Nadu. Animals were kept in standard animal house condition. Prior to use, the mice were housed in polypropylene cages in group of six animals under natural light-dark cycle. They were provided with commercial food pellets and tap water *ad libitum*. Cleaning and sanitation work was done on alternate days. Paddy husk was provided as bedding material. All the observations were made at room temperature in a noiseless diffusely illuminated room. The cages were maintained clean and all experiments were conducted between 8 am to 3 pm.

PROCEDURE:

Twelve animals Albino mice, (25-30gm) were selected for studies.

Most of the crude extracts possess LD₅₀, value more than 2000mg/kg of the body weight of the animal used. Dose volume was administered 0.1ml/100gm body weight to the animal by oral route.

After giving the dose toxic signs were observed within 3-4 hours. Body weight of the animals before and after administration, onset of toxicity and signs of toxicity like changes in the skin and fur, eyes and mucous membrane and also respiratory, circulatory, autonomic and central nervous systems activities, motor activity and behavior pattern, sign of tremors, convulsion, salivation, diarrhea, lethargy and sleep and coma was also to be noted, if any, was observed. The animal toxic or death was observed upto 14 days.

OBSERVATION

Acute toxicity studies and evaluation of data are studied as per the guideline of OECD (423).

No toxicity or death was observed for these given dose levels, in selected and treated animals. So the LD₅₀ of the Methanolic extract of leaves of *Syzygium aqueum* was greater than 2000mg/kg (LD₅₀>2000mg/kg).

Hence the biological dose was fixed at three levels, 125, 250 and 500mg/kg body weight for the extract.

5.4.2. EVALUATION OF ANXIOLYTIC AND ANTICONVULSANT ACTIVITY

Animals:

Albino mice of either sex 20-30 gm of body weight obtain from Animal House, Department of Pharmacology, J.K.K.M.M.R.F. College of Pharmacy, (Proposal No. – JKKMMRFCP/IAEC/2017/007) Namakkal DT, Tamil Nadu. Animals were kept in standard animal house condition. Prior to use, the mice were housed in polypropylene cages in group of six animals under natural light-dark cycle. They were provided with commercial food pellets and tap water *ad libitum*. Cleaning and sanitation work was done on alternate days. Paddy husk was provided as bedding material. All the observations were made at room temperature in a noiseless diffusely illuminated room. The cages were maintained clean and all experiments were conducted between 8 am to 3 pm.

Drugs and Chemicals:

- ✓ Diazepam (Calmpose Inj. Ranbaxy, India)
- ✓ Pentylenetetrazole (Sigma, USA)
- ✓ Methanol extra pure (S.D fine chemicals, Mumbai).

Experimental Design:

Animals are divided into 5 groups, each group containing 6 mice.

- Group I: Normal control mice fed with vehicle only.
- Group II: Mice treated with Diazepam 5mg/kg
- Group III: Mice treated with 125 mg/kg Methanolic extract of *Syzygium aqueum*
- Group IV: Mice treated with 250 mg/kg Methanolic extract of *Syzygium aqueum*
- Group V: Mice treated with 500 mg/kg Methanolic extract of *Syzygium aqueum*

5.4.2.1. EVALUATION OF ANXIOLYTIC ACTIVITY**5.4.2.1.1. Elevated Plus Maze (EPM) Test.**

The EPM test is the most frequently employed model for the assessment of the anxiolytic activity of novel substances (R.G.Liser 1987). The elevated plus maze apparatus consisted of two perpendicular open arms (50 X 10 cm) and two perpendicular enclosed arms (50 X 10 X 40 cm). The entire maze was constructed of wood and elevated 50 cm above floor. The maze was placed inside a light (25 lx) and sound attenuated room.

The animals were divided into five groups, each group comprised six mice. Different groups were treated with distilled water (10 mL/kg), diazepam (5 mg/kg), and Methanolic Extract of *Syzygium aqueum* at doses of 125, 250, and 500 mg/kg, BW. Thirty minutes later, the rat was placed in the center platform of the maze facing the enclosed arm and was observed for 10 min. The parameters assessed were the time spent in open and enclosed arms and numbers of open and enclosed arms entries. All tests were taped by using a video camera and every precaution was taken to ensure that no external stimuli could evoke anxiety in the mice. After each test, the maze was carefully cleaned up with a wet tissue paper (70% ethanol solution) to eliminate the interference of the olfactory cues on the next rat (Peng.W.H *et al.*,2000).

5.4.2.1.2. Open Field Test.

The study was conducted according to method previously described by Brown et al with some modifications. The apparatus was made up of plywood measuring 72 cm X 72 cm X 36 cm. One of the walls was made of transparent Perspex glass to ensure that the mouse under investigation is visible to the observer. The floor, made of cardboard, was divided into 16 equal squares (18 cm X 18 cm) with blue marker and a central square drawn with black marker. The cardboard was covered with a transparent Plexiglas. The animals were divided into five groups; each group comprised six rats. Different groups were treated with distilled water (10 mL/kg), diazepam (5 mg/kg), and Methanolic extract of *Syzygium aqueum* at doses of 125, 250, and 500 mg/kg, BW. Thirty minutes later, each mouse was placed individually at the corner of the arena and its behavior monitored for 5 min. The number of rearings and number of square crossed by each mouse was recorded.

The apparatus was wiped between observations with 70% ethyl alcohol and allowed to dry to remove any olfactory cue.

5.4.2.1.3. Rota rod:

The equipment of Rotarod was used to evaluate motor coordination produced by drugs in animals. The mice were trained before the experiment to acquire the capacity to remain for 300 s on a diameter rod, rotating at 20 rpm. Two or three trials were sufficient for the animals to learn this task. Thirty mice were divided into five groups; each group comprised six rats. Different groups were treated with distilled water (10 mL/kg), diazepam (5 mg/kg), and Methanolic extract of leaves of *Syzygium aqueum* at doses of 125, 250, and 500 mg/kg, BW. Then, the animals were placed in the four paws on the rotating bar, which is 2.5 cm in diameter and 25 cm high from the floor. The animals were observed for a period of five minutes. The difference between the fall-off time of the mice before and after treatment was considered as an index of muscle relaxation (Farkas.S *et al.*, 2005).

5.4.2. EVALUATION OF ANTICONVULSANT ACTIVITY

5.4.2.1. Pentylenetetrazole Induced Convulsions:

Pentylenetetrazole (PTZ) induced convulsions test was performed to evaluate anticonvulsant property of drugs (Ahmadiani.A *et al.*,).Thirty male mice were divided into five groups, each group comprised six mice. Different groups were treated with distilled water (10 mL/kg), diazepam (5 mg/kg), and Methanolic extract of *Syzygium aqueum* at doses of 125, 250, and 500 mg/kg, BW. Thirty minutes later, convulsions were induced by the intraperitoneal administration of 60 mg/kg BW of PTZ. Following the administration of PTZ, mice were placed in

separate transparent plexiglass cages (25 × 15 × 10 cm) and were observed for the occurrence of seizures over a 30 min time period. Latency of convulsions (the time prior to the onset of tonic convulsions), duration of tonic convulsions, and mortality protection (percentage of deaths in 24 h) were recorded (R.S. Fischer, 1989).

5.4.2.2. Maximal Electro Shock (MES) Induced Convulsions:

The animals were divided into five groups, each group comprised six mice. Different groups were treated with distilled water (10 mL/kg), diazepam (5 mg/kg), and Methanolic extract of *Syzygium aqueum* at doses of 125, 250, and 500 mg/kg, BW. Thirty minutes later, convulsions were induced in all the groups of animals using electro convulsimeter. A 60 Hz alternating current of 150 mA for 2 s was delivered through the ear electrodes (Balamurukan.G.*et al.*). The animal was observed for the occurrence of tonic hind limb extension.

5.5. Data analysis

Results of the experiments and observations were expressed as mean ± standard deviation (SD). The significance of differences between groups was determined using one-way analysis of variance (ANOVA) followed by at least one of the following post hoc tests: Dunnett's multiple comparison tests $P < 0.05$ where level of significance was considered for each test. The data is presented as mean ± S.D.

CHAPTER 6

RESULTS AND DISCUSSION

Based on literature review, the leaves of *Syzygium aqueum* of family Myrtaceae was collected, authenticated and the project was carried out. The result of the present study show that the Methanol extract of *Syzygium aqueum* leaves shows significant anticonvulsant and anxiolytic activities.

6.2 PRILIMINARY PHYTOCHEMICAL STUDIES

Table No. 6.1. Percentage Yield of *Syzygium aqueum*

| Name of extract | Yield(%w/w) |
|-----------------|-------------|
| Methanol | 7 |

The extract obtained were subjected to qualitative Phytochemical test to find out the active constituents.

Table No.6.2: Qualitative Phytochemical analysis of the extract

| TEST FOR PHYTOCONSTITUENTS | RESULT |
|--------------------------------|--------|
| Saponins | - |
| Alkaloids | + |
| Glycosides | + |
| Tannins and phenolic compounds | + |
| Carbohydrate | + |
| Terpenoids | + |
| Flavonoids | + |
| Steroids | + |

(+) - Present (-) - Absent

DISCUSSION:

The preliminary Phytochemical studies were done in the Methanolic extract of *Syzygium aqueum* leaves, the result suggest that presence of **Alkaloids, Carbohydrate, Terpenoids, flavonoids, Steroids, phenolic compounds and tannins.**

6.3 PHARMACOLOGICAL STUDIES**6.3.1 ACUTE ORAL TOXICITY STUDIES**

The acute oral toxicity of the Methanolic extract of *Syzygium aqueum* was carried out as per OECD 423-guidelines (Acute toxic class method). Acute toxicity studies revealed that $LD_{50} > 2000 \text{mg/kg}$ for the extract. Hence, the biological dose was fixed at 125, 250mg and 500mg/kg body weight.

6.3.2. EVALUATION OF ANXIOLYTIC ACTIVITY**Elevated plus maze:**

Administration of diazepam (5mg/kg) significantly increases number of open arm entries, time spent in open arms and the number of rearings in open arm. They showed a reduction in the time spent in closed arm. Plant extracts treated mice exhibited significant increase in the number of open arm entries. The number of arm entries, but decreases in time spent in closed arm as shown in the table 6.3.

Open field test:

There was significant anxiolytic activity observed with diazepam, plant extracts when compared to control. In the open field test, plant extract showed

significant increase in number of rearings, number of squares crossed and number of assisted rearings during 5 min intervals of test as compared with control as show in table 6.4.

Rota rod:

Table 6.5.shows the effects of Methanolic extract of leaves of *Syzygium aqueum* in the Rotarod test, a method used for evaluating motor coordination and presence of any muscle griping effect. It revealed that there was significantly increased grip force and fall time after administration of Methanolic extract of *Syzygium aqueum* (125, 250, and 500 mg/kg) when compared to control. All the plant extract treated animals retained on the rotating rod for more than 276.35 ± 7.58 s at 500 mg/kg as shown in Table 6.5 indicate the Methanolic extract of *Syzygium aqueum* to be devoid of neurotoxicity.

Table. 6.3. Effect of Methanolic extract of *Syzygium aqueum* on Elevated plus maze in mice

| Group | Treatment | Dose | Time spent in open arm (s) | Entries in open arm |
|-------|---------------|----------|----------------------------|---------------------|
| I | Saline | 10ml/Kg | 40.25±4.41 | 3.98±0.52 |
| II | Diazepam | 5mg/kg | 239.59±3.52** | 12.64±0.47** |
| III | Plant extract | 125mg/kg | 100.83±3.97 | 6.48±0.39 |
| IV | Plant extract | 250mg/kg | 173.81±4.32* | 6.53±0.42* |
| V | Plant extract | 500mg/kg | 213.92±4.80** | 10.32±0.21** |

The data represent the mean \pm S.D (n=6) *p<0.01, **p<0.001 significantly different compared to normal control and diazepam

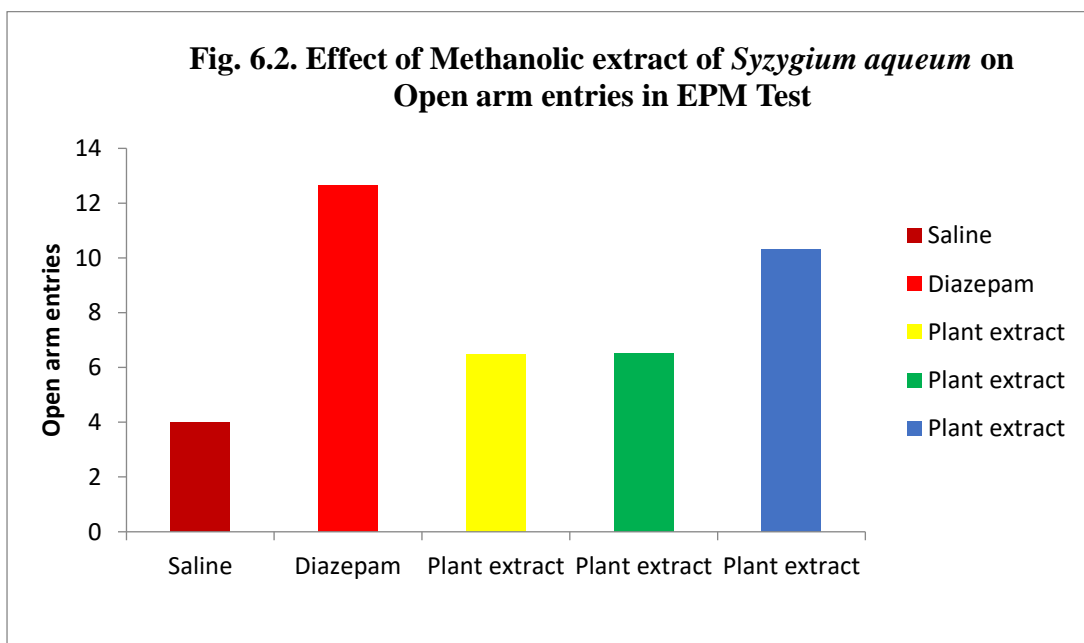
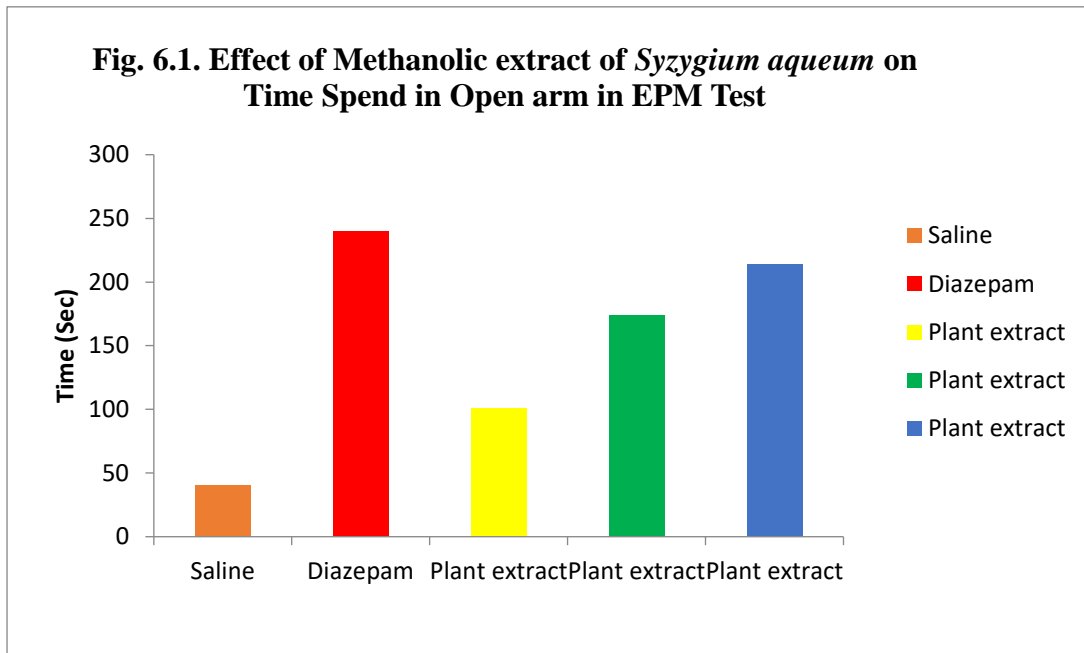


Table 6.4. Effect of Methanolic extract of *Syzygium aqueum* on Open field test in mice

| Group | Treatment | Dose | Number of square crossed | Number of rearing |
|-------|---------------|----------|--------------------------|-------------------|
| I | Saline | 10ml/kg | 40.3±2.1 | 10.1±1.4 |
| II | Diazepam | 5mg/kg | 29.5±3.6** | 8.2±1.8** |
| III | Plant extract | 125mg/kg | 22.9±2.4 | 5.4±2.3 |
| IV | Plant extract | 250mg/kg | 24.7±3.2* | 7.3±3.5* |
| V | Plant extract | 500mg/kg | 26.4±2.8** | 9.3±1.2** |

The data represent the mean ±S.D (n=6) *p<0.01, **p<0.001 significantly different compared to normal control and diazepam.

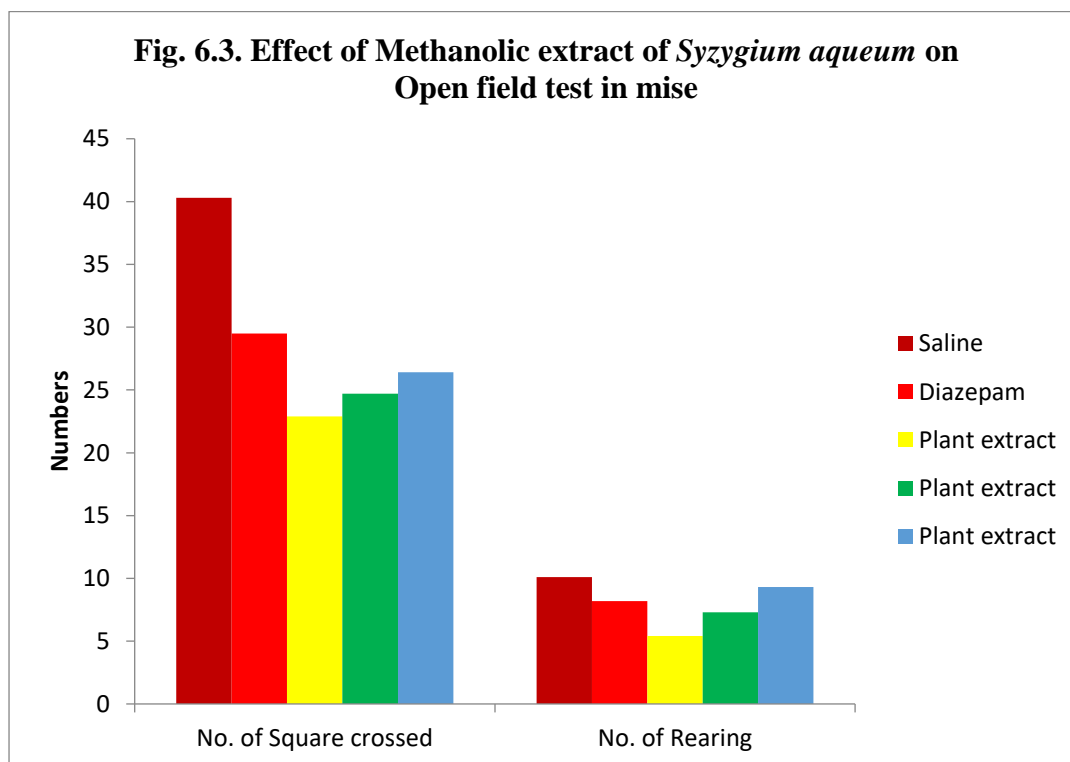
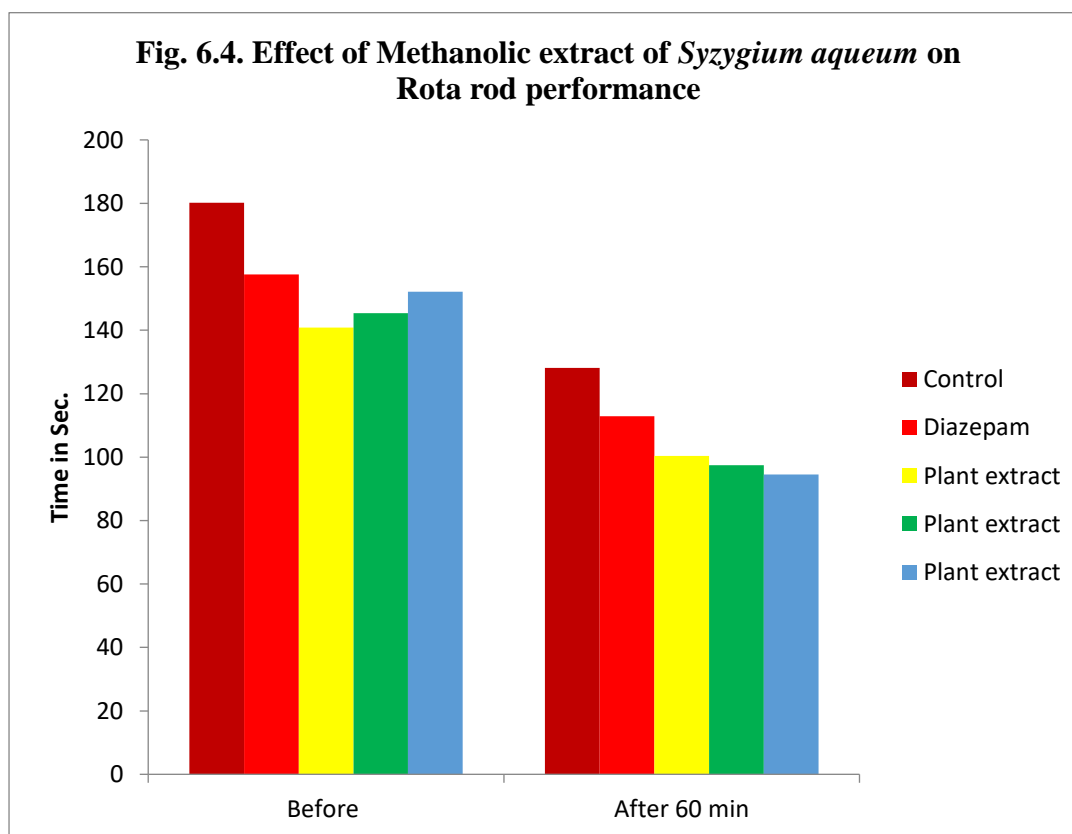


Table.6.5. Effect of Methanolic extract of *Syzygium aqueum* leaves on Rota rod performance

| Group | Treatment | Dose | Experimental mean time(10min) (s) | |
|-------|---------------|----------|-----------------------------------|--------------|
| | | | Before | After 60 min |
| I | Control | 10ml/kg | 180.21±9.1 | 128.10±7.6 |
| II | Diazepam | 5mg/kg | 157.61±6.9** | 112.90±6.3 |
| III | Plant extract | 125mg/kg | 140.82±5.7 | 100.39±7.2 |
| IV | Plant extract | 250mg/kg | 145.41±3.6* | 97.46±5.9 |
| V | Plant extract | 500mg/kg | 152.13±5.8** | 94.53±6.5 |

The data represent the mean \pm S.D (n=6) *p<0.01, **P<0.001 significantly different compared to normal control and diazepam.



6.3.3. EVALUATION OF ANTICONVULSANT ACTIVITY

6.3.1. PTZ Induced Convulsion:

Pentylentetrazole produced tonic seizures in the entire animals used. A dose of 125 mg/kg of Methanolic extract of leaves of *Syzygium aqueum* protected 33.33% of the animals against seizures and did not affect the onset (latency) of seizures to any significant extent. Methanolic extract of leaves of *Syzygium aqueum* at the dose of 250 and 500 mg/kg protected 50.0% and 100% of the mice against seizures and increased the latency of the seizures (Table 6.6).

6.3.2. Maximal Electro Shock Model:

Maximal electroshock produced hind limb tonic extension (HLTE) in all the animals. The vehicle treated mice showed tonic hind limb extension for duration of 12.88 ± 0.35 s. Administration of Methanolic extraction of leaves of *Syzygium aqueum* (125–500 mg/kg) showed a dose dependent increase in the delay of the onset time of seizures induced by maximal electroshock induced convulsion and also decreased duration of tonic hind limb extension (Table 6.7).

Table.6.6. Effect of Methanolic extract of leaves of *Syzygium aqueum* on PTZ induced convulsions in mice

| Group | Treatment | Latency of Tonic convulsion (s) | Duration of Tonic convulsions (s) | Mortality (% death) | % Protection |
|-------|--------------------------|---------------------------------|-----------------------------------|---------------------|--------------|
| I | Control | 100.20±3.34 | 446.10±5.19 | 6/6(100) | 0.0 |
| II | Diazepam (5mg/kg) | 478.34±6.07** | 126.69±1.93** | 0/6(0.0) | 100 |
| III | Plant extract (125mg/kg) | 141.43±1.98 | 216.29±1.23 | 4/6(66.66) | 33.33 |
| IV | Plant extract (250mg/kg) | 298.16±4.45* | 189.19±1.72* | 3/6(50.00) | 50.0 |
| V | Plant extract (500mg/kg) | 416.42±6.14** | 137.11±2.61** | 0/6(0.0) | 100 |

The data represents the mean S.D ± (n=6) *p<0.1, **p<0.001 significantly different compared to normal control and diazepam.

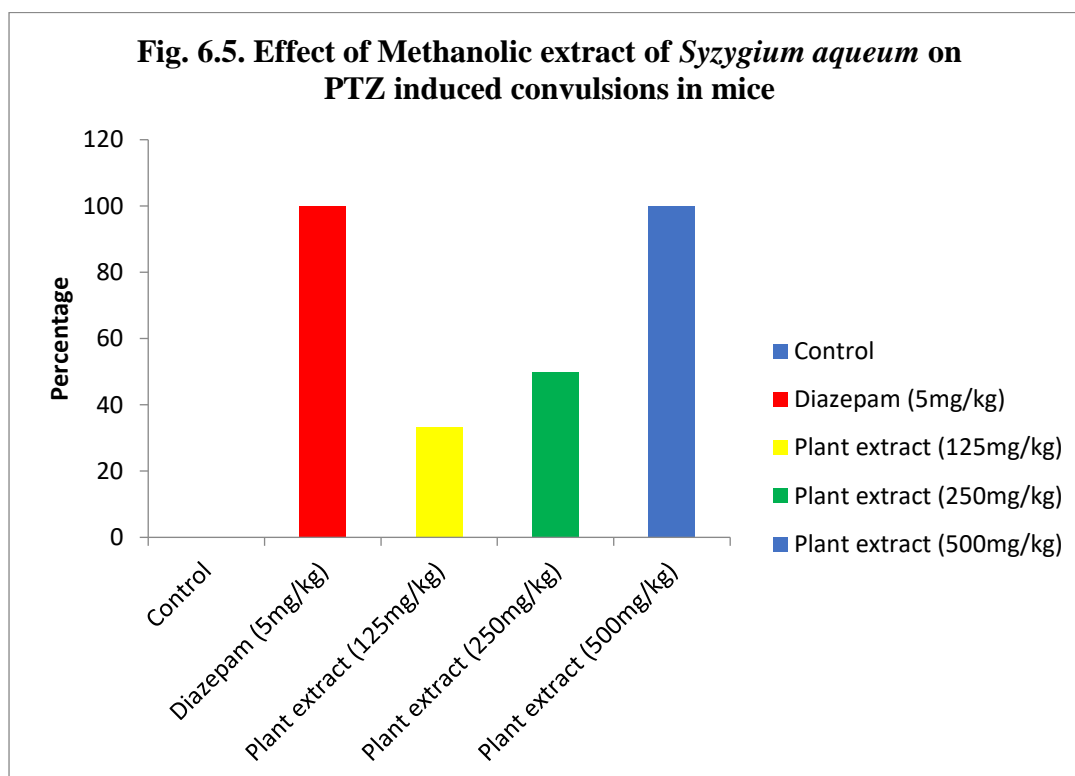
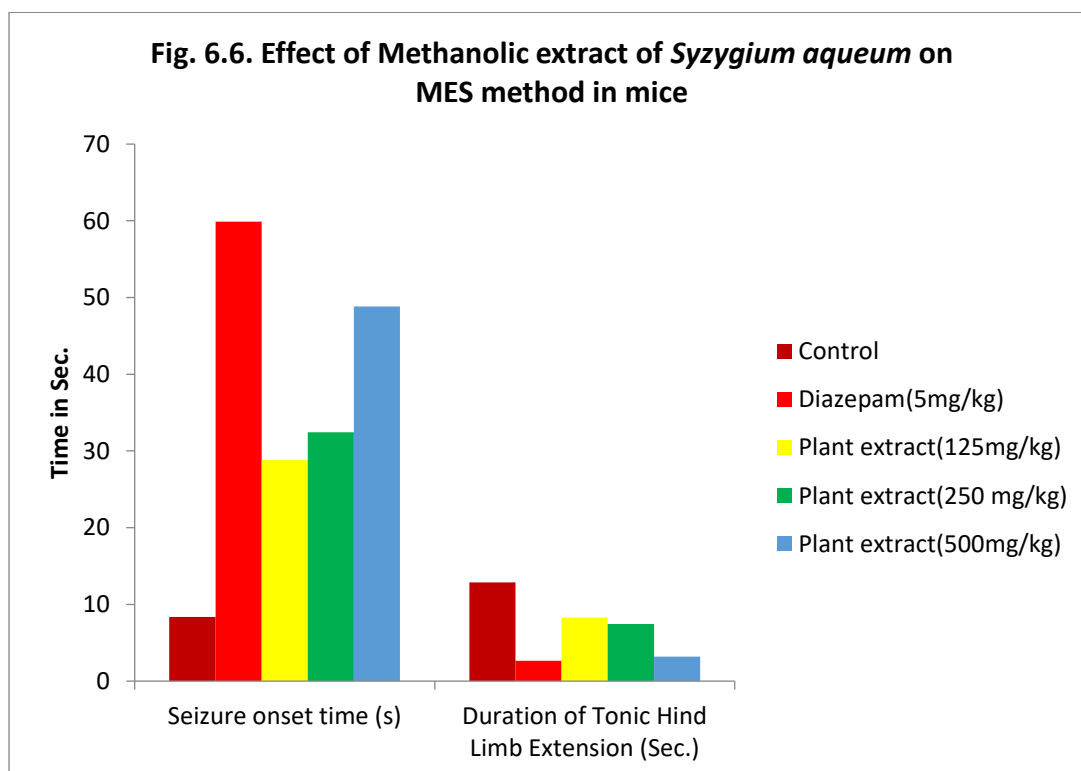


Table.6.7. Effect of Methanolic extract of *Syzygium aqueum* on Tonic seizures induced by MES method in mice

| Group | Treatment | Seizure onset time (s) | Duration of Tonic Hind Limb Extension (Sec.) |
|-------|--------------------------|------------------------|--|
| I | Control | 8.38±1.88 | 12.88±0.35 |
| II | Diazepam(5mg/kg) | 59.88±1.35** | 2.63±1.72** |
| III | Plant extract(125mg/kg) | 28.81±1.10 | 8.28±1.19 |
| IV | Plant extract(250 mg/kg) | 32.43±1.44* | 7.44±1.01* |
| V | Plant extract(500mg/kg) | 48.84±1.25** | 3.21±1.25** |

The data represent the mean ±S.D (n=6) *p<0.05, **p<0.001 significantly different compared to normal control and diazepam.



CHAPTER 7

SUMMARY AND CONCLUSION

The leaves of *Syzygium aqueum* belonging to family Myrtaceae has been examined to gain an insight of its Phytochemical and pharmacological behaviors.

The preliminary phytochemical investigation of Methanolic extract of leaves of *Syzygium aquem* showed the presence of **Carbohydrate, Alkaloids, Phytosteroids, Flavonoids, Phenolic compounds and Tannins.**

The pharmacological and acute toxicity studies of Methanolic extract was performed by following, OECD-423 guidelines (Acute toxic class method). No mortality or acute toxicity was observed upto 2000mg/kg of body weight.

Medicinal plants have served as sources of readily accessible, inexpensive, and effective medication since the earliest times known to man. Several ethnomedicinal plants have been found to possess neurobehavioral profile and serve as alternative to modern medicine. Biological evaluation and scientific validation of the ethnomedicinal plants are the need of the hour. The present study was proposed to assess anxiolytic, and anticonvulsant effects of methanolic extract of leaves of an ethnomedicinal plant, *Syzygium aqueum*.

Anxiety disorders are due to involvement of GABAergic, serotonergic, involvement. The adrenergic and dopamanergic system have also been shown to play a role in anxiety. BZA have been extensively, used for the last 40 years to treat several forms of anxiety, but due to their unwanted side effects, alternative treatment strategies with favorable side effect profiles. Medicinal plants are a good source to

find new remedies for these disorders. Despite the wide spread traditional use of *Syzygium aqueum* for treating various disorders there are no reports of scientific evaluation of its anxiolytic and anticonvulsant activity. The present work demonstrates that the *Syzygium aqueum* leaf extract had anxiolytic activity in mice by Elevated Plus Maze, Rotarod and Open field models.

Elevated Plus Maze is used to evaluate psychomotor performance and emotional aspects of rodents. Results showed that plant extracts treated mice exhibited significant increase in the number of open arm entries but decreases in time spent in closed arm, which reflects plants anxiolytic property.

The open field test is used to evaluate the animal emotional state. The open field model examines anxiety related behavior characterized by the normal aversion of the animal to an open area. Thus, animals removed from their acclimatized cage and placed in environment express anxiety and fear, by showing alteration in all or some parameters. Mice treated with extract showed increase in number of rearings and time spent in the center.

Rota rod test, the difference in the fall of time from the rotating rod between the vehicle and extract treated groups were taken as an index of muscle relaxation. Plant extract showed significant decrease in the locomotory score and fall of time of the mice from the rotating rod.

The results of the present laboratory animal study indicate that Methanolic extract of *Syzygium aqueum* leaf extract possesses anticonvulsant activity. The present study demonstrated the anticonvulsant effects of the methanolic extract of *Syzygium aqueum* in both chemically and electrically induced seizures in mice. The

extract exhibited dose dependent protection in the MES and PTZ induced convulsions. Nevertheless, in unprotected animals, the extract significantly increased seizure latency and reduced seizure duration compared with the control group in all two models at all tested doses. The effect of most of antiepileptic agents is to enhance the response to GABA by facilitating the opening of GABA-activated chloride channels. GABA receptors were involved in epilepsy and their direct activation would have an antiepileptic effect.

The anticonvulsant, anxiolytic, and sedative effects of benzodiazepines like diazepam are mostly attributed to enhance the action of gamma-aminobutyric acid (GABA_A) (Yemitan O.K. *etal.*,2005). Actually, benzodiazepines bind to the gamma subunit of the GABA_A receptor, due to which a structural modification of the receptor results in an increase in GABA_A receptor activity. Benzodiazepines do not substitute for GABA, which bind at the alpha subunit, but increase the frequency of channel opening events, which leads to an increase in chloride ion conductance and inhibition of the action potential (Muhammed.N *et al.*,and Garcia 2006). According to some researchers, the anxiolytic action of benzodiazepines may be due to the direct activation of glycine synapses in the brain (Muhammed.N *et al.*, and P.Brambilla, 2013). This may explain the mechanism of action of the tested extract as well, because it is clear from the results that the effect of the extract was similar to diazepam.

Previous phytochemicals reported in the literature, various Flavonoids, glycosides, Alkaloids and triterpenoids, isolated from *Syzygium aquem* would be the effective constituents for their anxiolytic and anticonvulsant effect.

In conclusion, Methanolic extract of *Syzygium aqueum* possesses anxiolytic and anticonvulsant effects and these findings collaborate with the ethnomedicinal uses of this plant. The isolation of active chemicals from this plant might serve as lead compounds for the synthesis of drugs which could be used in the management of these nervous disorders.

CHAPTER 8**BIBLIOGRAPHY**

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