STUDY OF ANXIOLYTIC ACTIVITY IN MICE USING VACHA CHURNAM (RASAYANA PREPARATION- AYURVEDIC SYSTEM OF MEDICINE)

Dissertation submitted to

THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY,

CHENNAI

In partial fulfillment for the award of the degree of

MASTER OF PHARMACY

IN

BRANCH IV – PHARMACOLOGY

Submitted By

MONICA SHETTY KPL
REGISTRATION No: 261525007

Under the guidance of

DR.P.MURALIDHARAN,M.Pharm.,PhD.

Department of Pharmacology

DEPARTMENT OF PHARMACOLOGY

C.L.BAID METHA COLLEGE OF PHARMACY

(AN ISO 9001-2008 CERTIFIED INSTITUTION)

CHENNAI– 600097

OCTOBER – 2017

Phone : 24960151, 24962592, 24960425
e-mail : clbaidmethacollege@gmail.com
Website : www.clbaidmethacollege.com

C.L. Baid Metha College of Pharmacy
An ISO 9001:2008 approved institution
Jyothi Nagar, Old Mahabalipuram Road,
Thorapakkam, Chennai - 600 097.

Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai.
Approved by Pharmacy Council of India, New Delhi, and
All India Council for Technical Education, New Delhi

Dr.P.Muralidharan,M.Pharm., Phd

Prof & Head

Department of Pharmacology

CERTIFICATE

This is to certify that Project entitled **Study Of Anxiolytic Activity In Mice Using Vacha Churnam (Rasayana Preparation- Ayurvedic System Of Medicine)** submitted by Register No: **261525007** in partial fulfillment of the course for the award of the degree of **Master of Pharmacy in Pharmacology.** It was carried out at the Department of Pharmacology in C.L. Baid Metha College of Pharmacy, Chennai-97 under my guidance during the academic year 2016-2017.

Place : Chennai

Date : **(Dr.P.MURALIDHARAN)**

Phone : 24960151, 24962592, 24960425
e-mail : clbaidmethacollege@gmail.com
Website : www.clbaidmethacollege.com

C.L. Baid Metha College of Pharmacy
An ISO 9001:2008 approved institution
Jyothi Nagar, Old Mahabalipuram Road,
Thorapakkam, Chennai - 600 097.

Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai.
Approved by Pharmacy Council of India, New Delhi, and
All India Council for Technical Education, New Delhi

Prof.Dr. GRACE RATHNAM, M.Pharm., Ph.D.,

Principal

CERTIFICATE

This is to certify that Project entitled **Study Of Anxiolytic Activity In Mice Using Vacha Churnam (Rasayana Preparation- Ayurvedic System Of Medicine)** submitted by Register No: **261525007** in partial fulfillment of the course for the award of the degree of **Master of Pharmacy in Pharmacology.** It was carried out at the Department of Pharmacology in C.L. Baid Metha College of Pharmacy, Chennai-97. Under the supervision of ***Professor Dr. P. Muralidharan*** during the academic year 2016 - 2017.

Place : Chennai

Date : **(Prof.Dr.GRACE RATHNAM)**

DECLARATION

Register No: **261525007** hereby declare that this dissertation entitled, **Study Of Anxiolytic Activity In Mice Using Vacha Churnam (Rasayana Preparation- Ayurvedic System Of Medicine)** has been originally carried out by me under the guidance and supervision of **Prof. Dr. P. Muralidharan, M.Pharm,. PhD,** Head of the department of Pharmacology C.L.Baid Metha College of Pharmacy, Chennai-97 for the academic year 2016-2017.This work has not been submitted in any other degree eat any other university.

Place : Chennai

Date : **Register No: 261525007**

ACKNOWLEDGEMENTS

I wish to express my sincere and heartfelt thanks to my esteemed guide always encouraging, enthusiastic and confident about the success of for this project. Professor**.** Dr. **P. Muralidharan**, M. Pharm, Ph. D., Department of Pharmacology. C .L. Baid Metha College of Pharmacy. It is his critical suggestions and logical thinking that could lead to the logical conclusion of the project. It has always been a pleasure for me to work under his guidance. Words are not adequate to express my gratitude, for his co-operation and help which made me to complete the project.

I am also thankful to my principal, **Dr. Grace Rathnam**, M. Pharm., Ph.D., C. L. Baid Metha College of Pharmacy for her valuable contribution and facilities provided to our project work.

I express my deep sense of gratitude and sincere thanks to **Mr. Clement Atlee M.Pharm** for timely provision of animals to carry out and complete this work.

I am also thankful to **Mr. Anandan, Mr. Nathan Ruban, Mr. Rajaram,** Lab Assistants C.L. Baid Metha College of pharmacy for their help throughout my project work.

I am thankful to **Mr. Bahadur,** senior security officer, C. L. Baid Metha College of Pharmacy for his excellent duty in allowing us in college holidays and weekends.

I cannot ignore the support and help which I continuously received from my dear friends **Ramya**, **Ashish**, **Harika**, **Keerthi**, **Vijay**, **Muthukumaran**, **Mathew**, **Keziah**, **Vinitha**, **Adlin** whose tireless help and encouragement helped me in completeting this project and also for achieving my goals.

My humble thanks to **Almighty**, who gave me strength and confidence all along.

MONICA SHETTY

TABLE OF CONTENTS

LIST OF TABLES

LIST OF GRAPHS

LIST OF FIGURES

ABBREVIATION

S. NO	ABBREVIATION	EXPANSION
1	%	Percentage
2	/	Per (or) or
3	+ve	Positive
4	<	Less than
5	>	More than
6	a.m	ante meridiem
7	AD	After Death
8	ATP	Adenosine Tri-Phosphate
9	BDZ	Benzodiazepine
10	C	Celcious
11	Ca	Calcium
12	cm	Centimeter
13	CNS	Central Nervous System
14	conc	Concentration
15	CPCSEA	Committee for the Purpose of Control And Supervision of Experiments on Animals
16	DA	Dopamine
17	DBH	Dopamine beta-hydroxylase

18	DRD	Dopa-Responsive Dystonia
19	DSM	Diagnostic and Statistical Manual of Mental Disorder
20	EDTA	Ethylene Diamine Tetra Acetic Acid
21	EEG	Electroencephalogram
22	FDA	Food and Drug Administration
23	g	Gram
24	Hcl	Hydrochloric acid
25	i.p	Intra Peritonial
26	IAEC	International AnimalmEthical Committee
27	ICD	Interntional classification of diseases
28	ICV	Intra Cerebro Ventricular
29	IQ	Intelligent Quotient
30	Kg	Kilogram
31	L	Litres
32	LTP	Long term potentiation
33	luc	Illuminance
34	M	Molarity
35	MAO_A	Monoamine oxidase
36	ml	milliliter

37	Mm	milli Molar
38	N	Normality
39	N,S,E,W	North, South, East, West
40	NA	Nor-Adrenaline
41	NADPH	Nicotinamide adenine dinucleotide phosphate
42	NC	North Carolina
43	NDRI	Norepinephrine Dopamine reuptake Inhibitor
44	NE	Norepinephrine
45	NET	Norepinephrine reuptake Inhibitor
46	NMDA	N-methyl-D-aspartate receptor
47	NRI	Norepinephrine reuptake inhibitor
48	ODT	Orally Disintegrating Tablets
49	OECD	Oraganisation for Economic Co-operation and Developement
50	p.o	Per os
51	Ph	Power of Hydrogen/ Potential of Hydrogen
52	ROS	Reactive Oxygen Species
53	SD	Sprague Dawley
54	Sec	Seconds
55	SEM	Standarad Error mean

56	SERT	Serotonin transporter
57	SNRIs	Serotonin–norepinephrine reuptake inhibitor
58	SOD	Super Oxide Dismutase
59	SR	Sustained Release
60	SSRIs	Selective serotonin reuptake inhibitor
61	Std	Standard
62	SW	South west
63	US	United States
64	USA	United States of America
65	W	Watts
66	WHO	World Health Oranganization
67	α	alpha
68	β	beta

1. INTRODUCTION

Anxiety is an emotion characterized by an unpleasant state of inner turmoil, often accompanied by nervous behavior, such as pacing back and forth, somatic complaints, and rumination[1]. It is the subjectively unpleasant feelings of dread over anticipated events, such as the feeling of imminent death.[2] Anxiety is not the same as fear, which is a response to a real or perceived immediate threat, whereas anxiety is the expectation of future threat. [3] Anxiety is a feeling of uneasiness and worry, usually generalized and unfocused as an overreaction to a situation that is only subjectively seen as menacing.[4] It is often accompanied by muscular tension, restlessness, fatigue and problems in concentration. Anxiety can be appropriate, but when experienced regularly the individual may suffer from an anxiety disorder.

They are a group of mental illnesses, and the distress they cause can keep you from carrying on with your life normally. For people who have one, worry and fear are constant and overwhelming, and can be disabling. But with treatment, many people can manage those feelings and get back to a fulfilling life[5].

Anxiety can be either a short term "state" or a long term "trait". Whereas trait anxiety represents worrying about future events, anxiety disorders are a group of mental disorders characterized by feelings of anxiety and fear. Anxiety disorders are partly genetic but may also be due to drug use, including alcohol, caffeine, and benzodiazepines (which are often prescribed to treat anxiety), as well as withdrawal from drugs of abuse. They often occur with other mental disorders, particularly bipolar disorder, eating disorders, major depressive disorder, or certain personality disorders. Common treatment options include lifestyle changes, medication, and therapy.

1.1 TYPES OF ANXIETY DISORDERS [6]

Anxiety disorders are classified according to the severity and duration of their symptoms and specific behavioral characteristics. Types of anxiety disorders include:

1. Generalized anxiety disorder (GAD)
2. Panic disorder
3. Phobias
4. Obsessive-compulsive disorder (OCD)
5. Post-traumatic stress disorder (PTSD)
6. Separation anxiety disorder (which is almost always seen in children)

1.1.1 GENERALIZED ANXIETY DISORDER

Generalized anxiety disorder (GAD) is the most common anxiety disorder. It affects about 5% of Americans over the course of their lifetimes.

It is characterized by:

- A more-or-less constant state of worry and anxiety, which is out of proportion to the level of actual stress or threat in one's life.
- The anxiety occurs on most days during a period of more than 6 months despite the lack of an obvious or specific stressor. (It worsens with stress, however.)
- Patients with GAD may experience physical symptoms (such as gastrointestinal complaints) in addition to, or even in place of, mental worries.
- People with GAD tend to be unsure of themselves, overly perfectionist, and conforming.

Given these conditions, a diagnosis of GAD is confirmed if three or more of the following symptoms are present (only one for children) on most days for 6 months:

- Being on edge or very restless
- Feeling tired
- Having difficulty with concentration
- Being irritable
- Having muscle tension
- Experiencing disturbed sleep

Symptoms can cause significant distress and impair normal functioning. To be classified as GAD, they should not be due to a medical condition, another mood disorder, or psychosis. GAD rarely occurs by itself. It typically occurs along with another type of anxiety disorder, depression, or substance abuse.

1.1.2 PANIC DISORDER

Panic disorder is characterized by periodic attacks of anxiety or terror (panic attacks). Panic attacks usually last 15 - 30 minutes, although residual effects can persist much longer.

Panic attacks can occur in nearly every anxiety disorder, not just panic disorder. In other anxiety disorders, however, there is always a cue or specific trigger for the attack. A diagnosis of panic disorder is made under the following conditions:

- A person experiences at least two recurrent, unexpected panic attacks.
- For at least a month following the attacks, the person fears that another will occur.

Symptoms:

During a panic attack a person feels intense fear or discomfort and experiences at least four or more of the following symptoms:

- Rapid heart beat
- Sweating
- Shakiness
- Shortness of breath
- A choking feeling or a feeling of being smothered
- Dizziness
- Nausea
- Feelings of unreality
- Numbness
- Either hot flashes or chills
- Chest pain
- A fear of dying
- A fear of going insane

Women may be more likely than men to experience shortness of breath, nausea, and feelings of being smothered. Men may be more likely than women to have sweating and abdominal pain. Panic attacks that include only one or two symptoms, such as dizziness and heart pounding, are known as limited-symptom attacks. These may be either residual symptoms after a major panic attack or precursors to full-blown attacks.

1.1.3 PHOBIC DISORDERS

Phobias, manifested by overwhelming and irrational fears, are common. In most cases, people can avoid or at least endure phobic situations, but in some cases, as with agoraphobia, the anxiety associated with the feared object or situation can be incapacitating.

AGORAPHOBIA:

Agoraphobia is described as fear of being in public places or open areas. (The term comes from the Greek word agora, meaning outdoor marketplace.) In its severest form, agoraphobia is characterized by a paralyzing terror of being in places or situations from which the patient feels there is neither escape nor accessible help in case of an attack. Consequently, people with agoraphobia confine themselves to places in which they feel safe, usually at home. The patient with agoraphobia often makes complicated plans in order to avoid confronting feared situations and places.

SOCIAL PHOBIA:

Social phobia, also known as social anxiety disorder, is the fear of being publicly scrutinized and humiliated and is manifested by extreme shyness and discomfort in social settings. This phobia often leads people to avoid social situations and is not due to a physical or mental problem (such as stuttering, acne, or personality disorders).

The associated symptoms vary in intensity, ranging from mild and tolerable anxiety to a full-blown panic attack. (Unlike a panic attack, however, social phobia is always directly related to a social situation.) Symptoms include sweating, shortness of breath, pounding heart, dry mouth, and tremor.

SPECIFIC PHOBIAS:

Specific phobias (formerly simple phobias) are an irrational fear of specific objects or situations. Specific phobias are very common. Most cases are mild and not significant enough to require treatment.

The most common specific phobias are fear of animals (usually spiders, snakes, or mice), flying (pterygophobia), heights (acrophobia), water, injections, public transportation, confined spaces (claustrophobia), dentists (odontiatophobia), storms, tunnels, and bridges.

When confronting the object or situation, the phobic person experiences panicky feelings, sweating, avoidance behavior, difficulty breathing, and a rapid heartbeat. Most phobic adults are aware of the irrationality of their fear, and many endure intense anxiety rather than disclose their disorder.

1.1.4 OBSESSIVE-COMPULSIVE DISORDER

Obsessive-compulsive disorder (OCD) is a condition marked by unwanted intrusive and repeated thoughts (obsessions) and behaviors (compulsions):

Obsessions are recurrent or persistent mental images, thoughts, or ideas. The obsessive thoughts or images can range from mundane worries about whether one has locked a door to bizarre and frightening fantasies of behaving violently toward a loved one.

Compulsive behaviors are repetitive, rigid, and self-directed routines that are intended to prevent the manifestation of an associated obsession. Such compulsive acts might include repetitive checking for locked doors or unlit stove burners or calls to loved ones at frequent intervals to be sure they are safe. Some people are compelled to wash their hands every few minutes or to spend inordinate amounts of time cleaning their surroundings in order to subdue the fear of contagion.

A critical feature in this disorder is an inflated sense of responsibility, in which the patient's thoughts center on possible dangers and an urgent need to do something about them.

Over half of patients with OCD have obsessive thoughts without the ritualistic compulsive behavior. Although they recognize that the obsessive thoughts and ritualized behavior patterns are senseless and excessive, they cannot stop them. OCD often accompanies depression or other anxiety disorders. Some patients find that their symptoms subside over time, while others experience a worsening of symptoms.

Symptoms in children may be mistaken for behavioral problems (taking too long to do homework because of perfectionism, refusing to perform a chore because of fear of germs). Children do not usually recognize that their obsessions or compulsions are excessive.

1.1.5 POST-TRAUMATIC STRESS DISORDER

Post-traumatic stress disorder (PTSD) is a severe, persistent emotional reaction to a traumatic event that severely impairs one’s life. It is classified as an anxiety disorder because of its symptoms. Not every traumatic event leads to PTSD, however. There are two criteria that must be present to qualify for a diagnosis of PTSD

The patient must have directly experienced, witnessed, or learned of a life-threatening or seriously injurious event.

The patient's response is intense fear, helplessness, or horror. Children may behave with agitation or with disorganized behavior.

TRIGGERING EVENTS:

PTSD is triggered by violent or traumatic events that are usually outside the normal range of human experience. War is a prime example. There is some evidence that events most likely to trigger PTSD are those that involve deliberate and destructive behavior (such as murder or rape) and those that are prolonged or physically challenging. Such events include, but are not limited to, experiencing or witnessing sexual assaults, accidents, military combat, natural disasters (such as earthquakes), or unexpected deaths of loved ones. PTSD may also occur in people who have serious illness and receive aggressive treatments or who have close family members or friends with such conditions.

SYMPTOMS:

There are three basic sets of symptoms associated with PTSD. They may begin immediately after the event or can develop up to a year afterward:

1.**Re-experiencing**. In such cases, patients persistently re-experience the trauma in at least one of the following ways: in recurrent images, thoughts, flashbacks, dreams, or feelings of distress at situations that remind them of the traumatic event. Children may engage in play, in which traumatic events are enacted repeatedly.

2.**Avoidance**. Patients may avoid reminders of the event, such as thoughts, people, or any other factors that trigger recollection. They tend to have an emotional numbness, a sense of being in a daze or of losing contact with their own identity or even external reality. They may be unable to remember important aspects of the event.

3.**Increased Arousal**. This includes symptoms of anxiety or heightened awareness of danger (sleeplessness, irritability, being easily startled, or becoming overly vigilant to unknown dangers).

1.1.6 SEPARATION ANXIETY DISORDER

Separation anxiety disorder almost always occurs in children. It is suspected in children who are excessively anxious about separation from important family members or from home. For a diagnosis of separation anxiety disorder, the child should also exhibit at least three of the following symptoms for at least 4 weeks:

- Extreme distress from either anticipating or actually being away from home or being separated from a parent or other loved one
- Extreme worry about losing or about possible harm befalling a loved one
- Intense worry about getting lost, being kidnapped, or otherwise separated from loved ones
- Frequent refusal to go to school or to sleep away from home
- Physical symptoms such as headache, stomach ache, or even vomiting, when faced with separation from loved ones

Separation anxiety often disappears as the child grows older, but if not addressed, it may lead to panic disorder, agoraphobia, or combinations of anxiety disorders.

The cause of anxiety disorders is a combination of genetic and environmental factors.[7] Risk factors include a history of child abuse, family history of mental disorders, and poverty. To be diagnosed symptoms typically need to be present at least six months, be more than would be expected for the situation, and decrease functioning. Without treatment, anxiety disorders tend to remain.[6]

1.2 RISK FACTORS

Risk factors for anxiety disorders depend in part on the specific disorder. General risk factors include:

1. **Gender:** With the exception of obsessive-compulsive disorder (OCD), women have twice the risk for most anxiety disorders as men.
2. **Age:** Phobias, OCD, and separation anxiety typically show up early in childhood, while social phobia and panic disorder often develop during the teen years.
3. **Traumatic Events:** Traumatic events can trigger anxiety disorders, particularly post-traumatic stress disorder.
4. **Medical Conditions:** Although causal relationships have not been established, certain medical conditions have been associated with increased risk of panic disorder. They include migraines, obstructive sleep apnea, mitral valve prolapse, irritable bowel syndrome, chronic fatigue syndrome, and premenstrual syndrome.

1.3 SIGNS AND SYMPTOMS [7]

- Excessive worry
- Sleep problems
- Irrational fears
- Muscle tension
- Chronic indigestion
- Stage fright
- Self-consciousness
- Panic
- Flashbacks
- Perfectionism
- Compulsive behaviors
- Self-doubt

1.4 PATHOPHYSIOLOGY & NEUROTRANSMITTERS INVOLVED IN ANXIETY:

1.4.1 GAMMA-AMINO BUTYRIC ACID:

There are different inhibitory neurotransmitters in the CNS; most of the abundant and important is Gamma-amino butyric acid (GABA). The role of the inhibitory neurotransmitter GABA has long been regarded as Centre for the regulation of anxiety and this neurotransmitter system is the main target of benzodiazepines and other anxiety related drugs used to treat anxiety disorders. [8]

The excitability states in all brain areas are mainly controlled by GABA and the ongoing level of neuronal activity is regulated by the equilibrium between excitatory inputs (mostly glutamatergic) and inhibitory GABAergic activity. If the balance swings towards GABA, then sedation, amnesia and ataxia appear. On the other hand, the mildest attenuation of the GABAergic system results in restlessness, insomnia, arousal, anxiety and exaggerated reactivity. [9]

Historically, the GABA system has been thought to play a role in anxiety disorders largely because of the effectiveness of the benzodiazepines, which are well known to act primarily on GABA receptors, in the management of anxiety. [10]

When there is binding of GABA with the GABA-A±benzodiazepine receptor complex, it acts as an agonist: inducing conformational changes, with which the permeability of the central pore to chloride ions gets increased. The resulting chloride flux hyperpolarizes the neuron, leads to reduction in its excitability and producing a general inhibitory effect on neuronal activity.

The anxiolytic effects of drugs that act on the GABA receptor provide some of the strongest evidence that GABA dysfunction underlies anxiety states. Agents such as the benzodiazepines, gabapentin, pregabalin, valproate, vigabatrin, tiagabine demonstrate clinically relevant anxiolytic effects. [11]

1.4.2 BENZODIAZEPINES:

Benzodiazepines have been widely used to manage anxiety disorders, from the short-term relief of anxiety symptoms to specific anxiety syndromes (i.e. GAD, phobia, PD).[12] Benzodiazepines augment the GABAergic inhibition via GABA-A receptors. [13] Benzodiazepine binding allosterically changes the receptor complex to increase the GABA efficiency, which enables the GABAergic circuits to produce a larger inhibitory effect.

There is established efficacy of Benzodiazepines in the treatment of GAD, for which a drug such as Diazepam is suitable. [14] Typical side effects of BZD include drowsiness and impairment of cognitive and motor function. [15]

Benzodiazepines can potentiate the sedative effects of alcohol and other centrally acting drugs. Respiratory depression has been reported in some patients receiving benzodiazepines with clozapine. Drug discontinuation, even with a slow taper, can cause troublesome withdrawal reactions and rebound anxiety, which may necessitate re starting treatment. [16]

1.4.3 GABAPENTIN:

Gabapentin was designed as a GABA analog that could penetrate the blood- brain barrier. Although the anxiolytic properties of gabapentin are likely to be linked to its effects on the GABA system, gabapentin has a high affinity for the α2δ subunit of presynaptic P/Q-type, voltage–sensitive Ca^{2+}channels modulates certain types of Ca^{2+} current, and the release of several monoamine neurotransmitters gets reduced [17]

In Pre-clinical studies, gabapentin demonstrated anxiolytic effects similar to that of the BZDs. [18] Gabapentin has shown to effectively ameliorate the symptoms of PD and social phobia in placebo-controlled clinical studies. The most commonly reported adverse effects in gabapentin-treated patients with these anxiety disorders are dizziness, dry mouth, headache, nausea and somnolence. [19]

Case reports have suggested the potential use of gabapentin in the management of symptoms of PTSD [20], GAD, refractory PD [21] and GAD and schizophrenia/ mood disorders with comorbid PD and OCD. The most common side effects noted in these reports were drowsiness and dizziness. [22]Abrupt discontinuation of augmentative gabapentin treatment has been found to lead to withdrawal symptoms such as rebound anxiety related symptoms of depression and sleep disturbances. [23]

1.4.4 PREGABALIN:

Pregabalin is another GABA analog with similarities to gabapentin [24]; it is functionally similar to gabapentin. Pregabalin is thought to act by increasing total GABA content in the brain via an undetermined mechanism. [25]

Pregabalin was found to induce anxiolytic-like effects in a dose dependent manner in a preclinical murine model of anxiety [26] and reduces the GAD symptoms and social phobia in randomized, placebo-controlled clinical trials.[27] the most commonly reports adverse events in pregabalin clinical trials were dizziness, somnolence, dry mouth, nausea and ataxia.

1.4.5 GLUTAMATE:

The excitatory action of amino acid L-glutamate in the mammalian brain and spinal cord has been known since the 1950s. [28] Glutamate is the main excitatory neurotransmitter in the human Central Nervous System (CNS). Glutamate is ubiquitous within the central nervous system and has been shown to play important roles in different brain functions, including

neurodevelopment (e.g., differentiation, migration and survival), [29] learning (e.g., long-term potentiation and depression), [30] acute neurodegeneration (e.g., cerebral ischemia, traumatic brain injury) [31] chronic neurodegeneration (e.g., Huntington's disease, Alzheimer's disease) [32] and, more recently, the stress response and anxiety disorders. [33]

Glutamate controls the synaptic release by a wide range of presynaptic receptors. These include not only the Group II and Group III glutamate metabotropic receptors but also cholinergic (nicotinic and muscarinic) receptors, adenosine (A1), kappa opioid, γ-amino butyric acid (GABA B), cholecystokinin and neuropeptide Y (Y2) receptors. [34]

Glutamate mainly show its actions through ligand-gated ion channel (ionotropic) receptors, including the N- methyl-d-aspartate (NMDA), kainate, and -amino- 3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) subtypes, and G protein-coupled metabo- tropic receptors (mGluR1-8). [35] Each NMDAR complex consists of four (occasionally five) subunits: two NR1 subunits (generated by alternative splicing of a single gene, NR1), and two or three NR2 subunits (coded by four related genes, NR2 A–D).

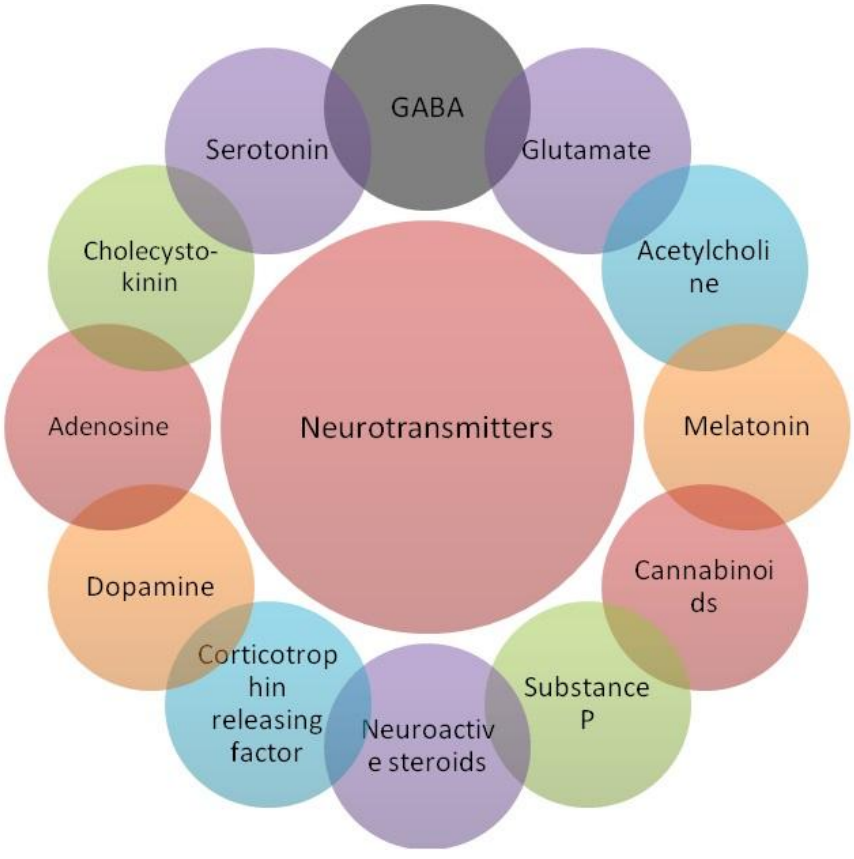


Fig. 1: Different neurotransmitters involved in anxiety

Recently, a third type of NMDA receptor subunit (NR3), which dramatically reduces ion permeability, has been described. [36]This situation leads to an enormous heterogeneity in terms of modulation of neurochemical profiles. [37]

Recently, a third type of NMDA receptor subunit (NR3), which dramatically reduces ion permeability, has been described. [36]This situation leads to an enormous heterogeneity in terms of modulation of neurochemical profiles. [37]

A major role is played by Glutamatergic system in the pathogenesis of anxiety and fear conditioning. Many studies suggest that glutamatergic neurotransmission of limbic system plays a pivotal role in the pathogenesis of anxiety disorders. [38]Severe stress exposure directly leads to glutamate excitotoxicity, which can cause neuronal damage and/or death. By decreasing the level of endogenously released glutamate, the anxiolysis could be induced. NMDA receptor would be activated by diminished glutamate release but to less extent and CNS excitation would remain at a stable stage. Such an effect can be achieved by switching on the regulatory machinery of presynaptic glutamate release. [39]

Monosodium glutamate (MSG) [$C_5H_8NO_4NaH_2O$] a sodium salt of naturally occurring (non-essential) L- form of glutamic acid, is one of the flavor enhancers, which is mainly used as an ingredient in various food products. [40] Glutamate Consumption has been linked to obesity and metabolic syndrome independent of physical activity and calorie intake. [41] Some researchers have also reported neurotoxic effects of glutamate. [42]

MSG is an excitotoxic which excites the neurons and may cause their death. Moreover, it has been reported that MSG is a neurotoxic substance, which is capable of producing degeneration of population of neurons, accompanied by pathological conditions, such as stroke, epilepsy, schizophrenia, anxiety, depression, Parkinson's disease, Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis. [43]This could be due to its well knownexcitotoxic effect, as MSG can cause overexcitotoxicity of neuronal cells to damage or death point, resulting in brain injury, mostly accompanied by oxidative stress. [44]Spermidine, spermine and putrescine, which are polyamines, are found in green vegetables, milk products, and meat. Polyamines are naturally occurring, ubiquitous, low molecular weight aliphatic polycations with nucleophilic centers, which are found at elevated levels in the brain.[45]

The polyamines are a group of aliphatic amines with a polycationic structure, carrying a positive charge on each nitrogen atom at physiological pH. [46]Polyamines can interact with several intracellular targets, including nucleic acids and enzymes, and exert several selective and complex actions on a variety of ion channels. [47]Among them, especially remarkable is the dual modulation of a-amino-3-hydroxy- 5-methyl-4-isoxazole propionate (AMPA) [48] and N-methyl-

D-aspartate (NMDA) receptors [49], which have been implicated in important plasticity events, such as learning and memory. [50]

Polyamines (125–250 nmol, i.c.v.) cause damage of hippocampus and learning impairment in rats at high doses [51] and also increase dizocilpine-induced impairment of a learning task in rats. [52] These studies show that high cerebral levels of polyamines are neurotoxic and impair learning and memory. [53]

Different studies suggest that antidepressant drugs (SSRIs, SNRIs, TCAs, and MAOIs) decreases glutamatergic activity in some regions (like hippocampus) on chronic administration [54] and that NMDA receptor antagonists on acute administration have antianxiety and antidepressant properties in preclinical and clinical models. [55] Glutamate release is inhibited by Lamotrigine and is currently used as an anticonvulsant as well as a treatment for bipolar depression. A clinical study has shown lamotrigine to be effective in certain PTSD symptoms (re-experiencing and avoidance/ numbing). [56] Similarly, topiramate, an AMPA/ kainate blocker among other actions, was found in an open-label study to reduce re-experiencing symptoms in PTSD. [57]

1.4.6 SEROTONIN:

5-HT plays a vital role in the development and the persistence of anxiety disorders in addition to GABA. Different studies have shown that 5-HT concentration when increased in the brain also increases anxiety and a reduction of 5-HT level reduces anxiety. [58] Serotonergic neurons are involved in the alteration of appetite, energy, sleep, mood and cognitive function in anxiety. Its role in anxiety is supported by its modulating effect on the locus coeruleus and its projections to the amygdale. Fear and stress activate serotonergic pathways. [59]

Several studies show that anxiety disorders patients may have genetic polymorphisms in the 5-HT transporter [60] or in the 5-HT2A receptor [61] and the 5-HT1A receptor. [62] Panic disorder patients show reduction in the number of 5-HT1A receptors in the limbic system [63] There is an involvement of 5-HT3 receptors in the regulation of anxiety [64] but clinical efficacy is still uncertain.[65]

SNRIs (Serotonin-Norepinephrine reuptake inhibitors) results in 5-HT and NA transporters binding to selectively inhibit the reuptake of these neurotransmitters from the synaptic clefts. SNRIs show “dual mode of action”. SNRIs block the reuptake of both 5-HT and NA with differing selectivity. Whereas milnacipran blocks reuptake of 5- HT and NA with equal affinity, duloxetine has a 10-fold greater selectivity for 5- HT, and venlafaxine show 30-fold greater selectivity for 5-H. [66]

Venlafaxine was the first SNRI that comes in market. Venlafaxine inhibits neuronal uptake of 5- HT (most potent, present at low doses), NA (moderate potency, present at high doses) and dopamine (DA) in order of decreasing potency. Venlafaxine has no affinity for α2- or β- adrenoceptors, benzodiazepine or opiate receptors. It has a much greater affinity for the 5- HT transporter than for the norepinephrine (NE) transporter. At low doses, it inhibits the 5- HT transporter almost exclusively, acting like a selective serotonin reuptake inhibitor (SSRI), with significant NE reuptake inhibition only occurring at higher doses. [67]

1.4.7 CHOLECYSTOKINNIN:

CCK, a neuropeptide, was, like 5-HT, discovered originally in the digestive tract and found in the CNS later. [68] CCK-immunoreactive fibers and CCK (2) receptors are most abundantly present in anatomical locations like periaqueductal gray (PAG), which mediate anxiety.[69] The CCK2 receptor regulates the fear-related behaviours in humans and animals [70] CCK-4 injection triggers the panic attacks in patients with a history of panic disorder. [71]

1.4.8 ADENOSINE:

Adenosine results due to hydrolysis of 5-adenosine monophosphate and is transformed to inosine, which is then stored as adenosine triphosphate. [72] Adenosine is also involved in the regulation of anxiety-related behavior. High doses of caffeine, which is the nonselective adenosine receptor antagonist, induce fear in healthy people and trigger panic attacks in anxiety disorder patients. [73] Adenosine through A1 and A2A receptors exert anxiolytic effect through its facilitatory influence on release of GABA in the septum and hippocampus.[74] On treatment with caffeine, rats were more anxious in the elevated plus-maze test (X-maze) [75] and a free exploratory paradigm, [76] while an adenosine-1 receptor agonist had an anxiolytic effect in the X-maze. [77]

1.4.9 ACETYLCHOLINE:

Acetylcholine plays a pivotal role in learning and memory processes. Acetylcholine levels can be modulated by stress in several brain regions. Acetylcholinesterase present in the CNS catalyzes the hydrolysis of acetylcholine to choline. [78] Acetylcholine is released in the synaptic cleft where it activates both presynaptic and postsynaptic cholinergic receptors namely nicotine and muscarinic leading to an increase of cholinergic transmission which results in cognitive impairment. [79] Cholinergic input to hippocampus is enhancing in response to anxiogenic and stressful stimuli.[80] Muscarinic M1 receptors induce anxiety through noradrenergic pathway. Nicotine facilitates GABAergic neuron that induces anxiety. [81]

1.4.10 CANNABINOIDS:

Cannabinoid receptor is widely distributed in the CNS present in the brain areas related to stress responses such as the central amygdala and the paraventricular nucleus of the hypothalamus and in the limbic system. Cannabinoid-1 agonist can induce both anxiolytic and anxiogenic responses in animal studies. Low doses of the cannabinoid produce anxiolytic effects, whereas higher doses result in anxiety. Acute administration of the selective CB1 receptor antagonist SR141716A induced anxiety-like responses in the elevated plus maze and the defensive withdrawal tests. CB1 knockout mice showed anxiogenic-like response in the light/dark box. [82]

1.4.11 CORTICOTROPHIN-RELEASING FACTOR:

Corticotrophin-Releasing Factor is made up of peptide containing 41 amino acids. This neurotransmitter in CNS acts as a key mediator of autonomic, immune, behavioral and endocrine stress responses. The peptide appears to be anxiogenic, proinflammatory and leads to increase pain perception. [83] Corticotrophin-Releasing Factor is an essential component, which mediates endocrine and behavioral anxiety-like responses, and stimulation of CRF2 may produce anxiolytic-like effects. [84]

1.4.12 MELATONIN:

Melatonin is synthesized by the pineal gland during night and acts through G-protein coupled receptors (GPCRs), MT_1 (MEL1a) and MT_2(MEL1b). Melatonin is involved in numerous physiologic processes including circadian rhythms, mood regulation, anxiety, sleep, appetite, immune responses and cardiac functions. Preoperative anxiolytic effects of melatonin found a significant reduction in anxiety. MT_2 receptors modulate anxiety levels and consequently this receptor may become a novel target for the treatment of anxiety. [85]

1.4.13 SUBSTANCE P:

Substance P neurotransmission has been associated with aversion and anxiety behavioral model. Substance P act primarily at the neurokinnin-1 (NK1) receptor. NK-1 antagonists are mediated by the dorsal raphe nucleus. NK-1 receptor exhibit anxiolytic effects in several models including elevated plus maze and social interaction tests. Disruption of NK-1 receptor results in 5-HT_{1A}-receptor desensitization and anxiolytic behavior and the effect of substance P play a role in anxiogenic behavior. [86]

1.4.14 NEUROACTIVE STEROIDS (NAS):

GABA-A receptor is a direct binding site for the neuroactive steroids. Enzymes involved in the biosynthesis of these NASs, such as 5α- reductase and 3α-hydroxy-steroid oxidoreductase are found in key neuroanatomic structures involved in the anxiety such as the amygdala and the hippocampus. Positive modulation of the GABA-A receptor has been associated with anxiolytic activity whereas anxiogenic activity in animals models of anxiety in association with negative modulation. The potential role of NAS analogues is in the treatment of anxiety disorders.

1.5 DIAGNOSIS OF ANXIETY[87]

Diagnosis is made using the Diagnostic and Statistical Manual of Mental Disorders IV (Text Revision) also called DSM-IV-TR.

The manual lays down criteria for diagnosis of each of the types of anxiety disorders. If these criteria are fulfilled for at least 6 months, the diagnosis may be made. Since anxiety disorders often coexist with other psychiatric disorders, diagnosis may be a challenge. For example, nearly 60% of patients with generalized anxiety disorders have accompanying panic disorder or depressive disorders.

1.5.1 DSM IV-TR CRITERIA FOR GENERALIZED ANXIETY DISORDER

The DSM IV-TR Criteria for Generalized Anxiety Disorder include:

- Presence of excessive anxiety about events or activities occurring on most days for at least 6 months
- Losing control over the worry intensity
- At least three of the symptoms including restless or jumpiness, fatigue, lack of concentration, irritability, muscle tension and sleep problems
- Significant interference of symptoms with social and work related functioning or leading to significant distress
- No other mood disorder or psychiatric problem

1.5.2 DSM IV-TR CRITERIA FOR PANIC DISORDER

The DSM IV-TR criteria for Panic Disorder include:

Frequent panic attacks without cause or warning. There may be presence of agoraphobia (fear of large open spaces). There are no other psychiatric or medial ailments that explain the attacks.

At least a single attack is followed by fear of:

- Fear and concern regarding another attack
- Worry regarding the consequences of an attack
- Change in behavior with relation to the panic attacks

1.5.3 DSM IV-TR CRITERIA FOR PTSD

The DSM IV-TR criteria for Post-traumatic stress Disorder include:

PTSD patients have a history of experiencing, witnessing or confronting an event that involved treat or actual risk of death or serious harm. The experience may be accompanied with feelings of fear, helplessness or horror. The feelings of distress persist for at least 1 month.

On presentation the patient may re-live the event by:

- Recurrent recollections of the event with thoughts, perception or flashes of images
- Recurrent dreams
- Sense of reliving the incident with illusions, hallucinations and flashbacks
- Severe psychological distress on exposure to cues to the event and a physiological reaction to the cues

Patient avoids or feels at least three of the following:

- Thoughts, feelings and conversation associated with the event
- Activities, places, or people associated with the event
- Loss of recall of the event
- Decreased interest in significant activities
- Detachment or estrangement from others

There may be associated symptoms of anxiety like:

- Sleep problems
- Irritability and anger outbursts
- Lack of concentration
- Increased vigilance
- Increased jumpiness or startle response
- Interference with social and work functioning

1.5.4 DSM IV-TR CRITERIA FOR OBSESSIVE COMPULSIVE DISORDER

The DSM IV-TR Criteria for Obsessive compulsive Disorder include :

- Obsessions include recurrent and intrusive thoughts, impulses or ideas. There are usually no excessive worries about real-life problems. Patient has attempted to ignore or suppress such thoughts and recognizes that the obsessional thoughts are a product of his or her own mind.
- Compulsions are repeated behaviors and mental actions that the patient is driven to follow according to self-set rigid rules. The compulsions ease the anxiety and reduce distress. These are not realistic and are clearly excessive.
- The compulsions may be recognised as excessive and may take over 1 hour a day.

1.5.5 DIAGNOSING CHILDREN WITH ANXIETY

Diagnosing children with an anxiety disorder is difficult. Anxiety in children may manifest as behavioral problems or as a disruptive or rebellious nature.

1.5.6 EXCLUSION OF MEDICAL CONDITIONS

Exclusion of medical conditions:

- Heart disease – Since chest pain and shortness of breath are common symptoms, heart disease and heart attacks should be ruled out. Mitral valve prolapse is a disorder where the mitral valve that lies between two chambers of the heart does not close well. This leads to impaired blood flow from the heart and back flow into the left atrium.There may be symptoms like chest pain, difficulty breathing especially after exercise, fatigue, cough, palpitations etc. this needs to be ruled out.

- Asthma – Panic attacks may mask asthma attacks
- Over active thyroid may lead to palpitations and needs to be ruled out.
- Low blood sugar or hypoglycaemia manifests as sweating and palpitations.
- Substance abuse and caffeine over dose may lead to anxiety and similar symptoms.

1.6 ANXIETY DISORDER STATISTICS[88]

Anxiety is a life altering condition. It's one that can affect the way you think, the way you feel, and the way you live your life. Unfortunately, anxiety is also incredibly common, affecting millions of people in the United States and millions more around the world.

This article will explore some key anxiety statistics. But the most important statistic you need to know is this: according to the National Institute of Mental Health (NIMH), 86% of those with anxiety disorders either do not seek treatment, or use treatments that are inadequate for stopping anxiety.

1.6.1 LIFETIME PREVALENCE

While year to year statistics are interesting, lifetime prevalence is the most important statistic in terms of the number of people that suffer from anxiety. 12 month prevalence statistics only show the number of those living with anxiety now, but your chances of developing anxiety at some point in your life are based on previous lifetime prevalence data.

The following is the likelihood of developing an anxiety disorder at some point in your life. Note that this is data on adults only.

- Any Anxiety Disorder: 28.8%
- Generalized Anxiety Disorder: 5.7%
- Obsessive Compulsive Disorder: 1.6%
- Panic Disorder: 4.7%
- Post-Traumatic Stress Disorder: 6.8%
- Social Phobia: 12.1%
- Specific (Other) Phobia: 12.5%

Every individual country has its own statistics, but they are likely to be somewhere near these overall numbers. Most other countries do not diagnose anxiety the same way as they do in the United States, nor do they adhere to the same measurements.

These numbers also do not necessarily represent the only factors at play for whether you will develop anxiety. Genetics plays a strong role, especially with panic disorder, so if your family members have panic attacks you'll increase your chances of getting it too.

1.7 ANTI- ANXIETY

An anxiolytic (also antipanic or anti anxiety agent) [89] is the medication or other intervention that inhibits anxiety. This effect is in contrast to anxiogenic agents, which increase anxiety. Together these categories of psychoactive compounds or interventions may be referred to as anxiotropic compounds or agents. Some recreational drugs such as alcohol (also known formally as ethanol) induce anxiolysis initially; however, studies show that many of these drugs are anxiogenic. Anxiolytic medications have been used for the treatment of anxiety disorder and its related psychological and physical symptoms. Anxiolytics have been shown to be useful in the treatment of anxiety disorder. Light therapy and other interventions have also been found to have an anxiolytic effect. [90]Beta-receptor blockers such as propranolol and oxprenolol, although not anxiolytics, can be used to combat the somatic symptoms of anxiety such as tachycardia and palpitations. [91]

Anxiolytics are also known as minor tranquilizers.[92]The term is less common in modern texts and was originally derived from a dichotomy with major tranquilizers, also known as neuroleptics or antipsychotics. There are concerns that some GABAergics (pertaining to or affecting the neurotransmitter GABA), such as benzodiazepines and barbiturates, may have an anxiogenic effect if used over long periods of time. [93]

1.8 DRUG CLASSIFICATION:

1. Barbiturates

- Phenobarbital
- Primidone

2. Benzodiazepines

- Alprazolam (Xanax)
- Bromazepam (Lectopam, Lexotan)
- Chlordiazepoxide (Librium)

- Clonazepam (Klonopin, Rivotril)
- Clorazepate (Tranxene)
- Diazepam (Valium)
- Flurazepam (Dalmane)
- Lorazepam (Ativan)
- Oxazepam (Serax, Serapax)
- Temazepam (Restoril)
- Triazolam (Halcion)

3. Carbamates

- meprobamate
- tybamate
- lorbamate.

4. Antihistamines

- Chlorphenamine
- Diphenhydramine

5. Opioids

- buprenorphine

6. Antidepressants

- Mirtazapine
- imipramine
- amitriptyline
- nortriptyline
- desipramine

7. Sympatholytics

- Beta blockers
- Alpha blockers
- Alpha-adrenergic agonist

8.Miscellaneous

- Mebicar
- Fabomotizole
- Selank
- Bromantane
- Emoxypine
- Azapirones
- Pregabalin
- Menthylisovalerate
- Propofol
- Racetams

1.9 MECHANISM OF ACTION OF ANXIOLYTICS[94]

Drugs to reduce anxiety have been used by human beings for thousands of years. One of the first anxiolytics and one that continues to be used by humans is ethanol. A detailed description of ethanol's action may be found in Chapter 100. A number of other drugs including the barbiturates and the carbamates (meprobamate) were used in the first half of the 20th century and some continue to be used today. This chapter focuses on current drugs that are used for the treatment of anxiety and approaches that are currently under investigation

- CORTICOTROPIN-RELEASING FACTOR (CRF
- GABAA RECEPTOR MODULATORS (BENZODIAZEPINES AND RELATED DRUGS)
- SEROTONIN RECEPTOR MODULATORS AND
- REUPTAKE INHIBITORS
- NEUROKININ RECEPTOR ANTAGONISTS
- GLUTAMATE RECEPTOR AGONISTS AND MODULATORS

1.9.1 GABAA RECEPTOR MODULATORS (Benzodiazepines and related drugs)

A majority of the synapses in the mammalian CNS use the amino acids l-glutamic acid, glycine, or -aminobutyric acid (GABA) for signaling. GABA is formed by the decarboxylation of l-glutamate, stored in neurons, and released, and its action is terminated by reuptake; GABA's action mimics the naturally occurring inhibitory transmission in the mammalian nervous system. Because of these findings, it has been accepted for over 20 years that GABA fulfills the characteristics of a neuro transmitter. Along with l-glutamate, acetylcholine, and serotonin, GABA possesses two different types of receptor conserved across different species and phyla that control both excitation and inhibition.

Molecular biological studies of the receptors causing these effects have indicated that GABA's effects on ionic transmission (ionotropic) and metabolism (metabotropic) are mediated by proteins in two different super families. The first superfamily (GABAA receptors) is a set of ligand-gated ion channels (ligand-gated superfamily) that convey GABA's effects on fast synaptic transmission. When a GABAA receptor is activated, an ion channel is opened (gated) and this allows chloride to enter the cell; the usual result of chloride entry is a slowing of neuronal activity through hyperpolarization of the cell membrane potential.

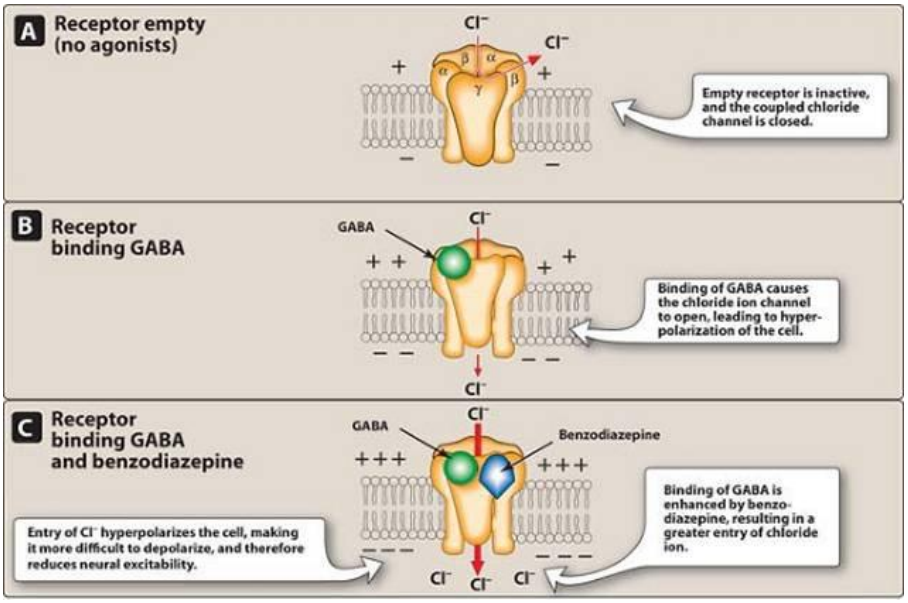


Fig. 2: Mechanism involved in anxiety

The second superfamily (GABAB) is slower, mediating GABA's action on intracellular effectors through a seven transmembrane spanning receptor (serpentine superfamily) that modulates the action of certain guanine nucleotide binding proteins (G proteins). Through their activity on other effector systems, G proteins can change second messenger levels, altering signal transduction and gene expression, or open ion channels that are dependent on the G-protein subunit activities. Both excitatory and inhibitory activities are possible on a time scale that is longer than GABAA receptor mediated events. There is extensive heterogeneity in the structure of the GABAA receptor members of the ligand-gated superfamily.

These receptors are the targets of a number of widely used and prescribed drugs for sleep, anxiety, seizure disorders, and cognitive enhancement; they may also contribute to mediating the effects of ethanol on the body.

1.10 ADVERSE DRUG REACTIONS[95]

Anxiety drugs are extremely tempting, and prescribed to millions of individuals suffering from anxiety and stress. Contrary to popular belief, these drugs aren't necessarily as harmful as many people believe. They have their problems, but there are those that do benefit from pharmaceutical intervention - especially if they are combining that medication with some type of therapy.

1.10.1 THE MAIN REASON TO AVOID ANXIETY DRUGS

Anxiety medicines do have some severe side effects in some cases. But it's actually not the side effects that are the reason to try to avoid medications. The main reason is because they can cause physiological and psychological dependence. This is when your body and mind actually start to need to drug to cope with anxiety, so that it becomes even harder to cure the anxiety without medication over time.

1.10.2 COMMON SIDE EFFECTS

Clumping all anxiety drugs together is misleading. Some have more side effects than others, just like some are stronger than others. Benzodiazepines can have some fairly pronounced side effects, while buspirone generally has fewer, but buspirone is also much weaker which is why most doctors prefer to prescribe benzodiazepines.

Even within the benzodiazepine class there are different side effects for different medications. But the most common side effects of anxiety medicines are as follows:

1. Benzodiazepines (Xanax, Valium, etc.):

- Hypotension (low blood pressure)
- Decreased sex drive/libido
- Nausea
- Lack of coordination
- Disinhibition
- Depression
- Unusual emotional dysfunction, including anger and violence
- Memory loss
- Difficulty thinking
- Decreased IQ

2. Antidepressants (SSRIs, SNRIs):

- Sexual dysfunction

- Headache
- Dizziness/drowsiness
- Diarrhea
- Weight loss/gain

Both drugs may also cause what's known as "paradoxical effects," where in some cases the person may experience a worsening of symptoms rather than an improvement. In some, this may be more anxiety. In others, this can even be severe suicidal ideation and possibly even action. This is uncommon, but still a serious concern.

1.10.3 DANGEROUS SIDE EFFECTS

Beyond the paradoxical effect of suicidal ideation and increased anxiety, it's rare for these drugs to show signs of truly dangerous and deadly side effects. But there are some rare cases of very dangerous problems from these medications, including:

- Heart problems, especially in those with possible cardiovascular disease.
- Persistent pulmonary hypertension (possibly fatal lung disease).
- Increased bleeding risk.
- Interactions with other medications.

Most of these are related to SSRIs. Benzodiazepines may also cause what's known as "dependency," which is when the body adapts to the anxiety drug. Dependency causes two issues. First, the medication will generally stop working (aka, "become tolerated") which means that you will need to find a replacement medication.

Second, weaning off the drug incorrectly can lead to severe withdrawal symptoms. These withdrawal symptoms are not unlike withdrawing from severe alcoholism, and may lead to increased anxiety, depression, psychosis, seizures, hypersensitivity, and possibly suicide. Gastrointestinal problems and insomnia are also very common. Withdrawal can be extremely dangerous, which is why benzodiazepines often need to be slowly weaned off of over time.

1.10.4 LONG TERM IMPLICATIONS

Anxiety drugs may have been given a worse reputation than they deserve. They're certainly not useless, and while they can cause some severe side effects, they are generally at least mildly well tolerated with most symptoms being distressing, but not terribly severe.

But when you combine the side effect risks with the physiological and psychological dependence risk, and the likelihood that you'll actually lose even more of your ability to cope with stress in the future if you depend on them, it's strongly recommended that anxiety

medications are used only as a last resort. If you do decide to take a medication, never take it alone - make sure you're also using long term anxiety reduction strategies as well so that if you stop taking the medication your anxiety doesn't return stronger than before.

1.11 NEW ANXIETY MEDICATIONS IN DEVELOPMENT [96]

The below new anxiolytics on the horizon that may be more effective and safer than older options.

1. ALORADINE (PH94B) [97]

This is a drug that has been in Phase III clinical trials since 2013 for the treatment of social anxiety disorder in women. It is being developed by the company Pherin Pharmaceuticals in the format of a "nasal spray." Of all the treatments for anxiety disorders in the pipeline, this one appears to have the most promise of actually making it to market.

The company developing this substance is known as Pherin Pharmaceuticals and focuses specifically on working with "pherins" to alter neuropsychiatric and neuroendocrine function within humans. They have other molecules in development for the treatment of depression, premenstrual dysphoria, and cognitive enhancement.

2. B-GOS

This is a prebiotic, or non-digestible food ingredients that improve healthy by stimulating the production of beneficial bacteria in the colon. It has long been thought that a person's gut flora (or bacteria) may play a role in determining whether they develop a mental illness or experience anxiety. Additionally, there is evidence demonstrating that most pharmaceutical drugs (e.g. antidepressants) have detrimental effects upon a person's gut flora.

Clasado Biosciences Limited currently are testing "B-GOS" in Phase I clinical trials. B-GOS is considered a unique "trans-galactooligosaccharide" and thus far has been shown to be effective in the reduction of anxiety. In preliminary research, it has been found to decrease both "waking cortisol levels" as well as "attentional vigilance towards negative information."

Some speculate that this substance may also prove to have therapeutic effects among individuals suffering from depression. There is mounting evidence that gut bacteria may be directly influencing brain function. Altering the gut microbiome may drastically change your mental health and/or cognition.

3. IW-2143 (BNC210)

This is an experimental anxiolytic medication undergoing clinical trials, but there's currently no information regarding its mechanism of action. It has demonstrated a potent anxiolytic effect in animal subjects as well as in Phase I clinical trials. The good news is that it is unlikely to impair cognitive function, it isn't addictive (in rodent models), and won't make you feel sedated. No major side effects have been reported during the early stages of trials.

This drug is a "small molecule" that was discovered by the company Bionomics through a medicinal-chemistry program. Some evidence has shown that the molecule is capable of promoting "neurite" outgrowth (e.g. axon/dendrite). The company developing this drug (Bionomics) is a biotech company that is focused on creating therapies for CNS disorders. They have since licensed "BNC210" to Ironwood Pharmaceuticals, who refer to it as "IW-2143."

4. S32212 [98]

This is a substance that is under investigation as a potential antidepressant with anxiolytic properties. It functions as a selective, inverse agonist at the 5-HT2C receptor, and Alpha-2 adrenergic receptor antagonist. It also elicits an effect upon the 5-HT2A receptor and a minimal one on the 5-HT2B receptor as an antagonist.

The good news is that it is thought to not affect histamine or acetylcholine receptors. Many drugs that affect H1 histamine and mACh receptors tend to result in memory impairment. Should this drug ever make it to the market, it will likely be classified as an NaSSA (noradrenergic and specific serotonergic antidepressant); the same classification of Remeron.

Based on animal research, this drug elevated levels of BDNF with the amygdala and hippocampus of the brain. It also amplified the amount of neuronal firing within other locations, while increasing neurotransmitters like norepinephrine, dopamine, and acetylcholine (in the prefrontal cortex). It didn't affect serotonin or histamine (which is probably a good thing).

Researchers have noted a variety of effects resulting from this substance including: antidepressant, anxiolytic, anti-obsessional, as well as anti-aggressive behaviors. It also is thought to enhance sleep and cognition in animals without any signs of weight fluctuation or diminished sex drive. Some speculate that it may even result in weight loss due to its "alpha blocking" effect.

5. SL-651,498

This is a drug that has long been used as an anxiolytic in scientific research, but wasn't considered for human usage until 2006. In 2008, a report surfaced that preliminary human trials were underway and that the drug was considered as effective as Ativan in reducing anxiety, yet produced minimal sedation, cognitive impairment, or reduction in motor skills. This provides some early evidence that SL-651,498 may be an effective treatment.

Although its anxiolytic potential is equal to that of benzodiazepines, it is structurally distinct from the benzo classification. It has been classified as a "nonbenzodiazepine anxiolytic." It functions as a subtype-selective GABAA agonist, and elicits primarily anxiolytic effects in animals (with minor sedation). Early research has also suggested that the drug is unlikely to produce dependence or tolerance due to its low affinity for the Alpha-5 receptor.

1.12 HERBAL REMEDIES FOR ANXIETY[99]

Plants parts - seeds, berries, roots, leaves, bark, and flowers have been used medicinally by every culture. Archaeologists have found evidence that we've been using plants medicinally for at least 60,000 years! Eighty percent of the world's population still relies on herbal remedies as part of their primary health care. There's been resurgence in the use of herbs for anxiety in recent years. This interest has been fueled by the rising costs of prescriptions plus the awareness that anti-anxiety drugs like Xanax and Ativan have serious side effects, including being some of the most addictive substances known.

1. ARCTIC ROOT (Rhodiolarosea)

Arctic root, as the name suggests, is found mainly in cold regions of the world like the Arctic and mountains of central China. It's a potent adaptogen, a substance that strengthens your overall resistance to both physical and emotional stress.

This makes Arctic root particularly useful for anxiety accompanied by fatigue.

2. ASHWAGANDHA (Withaniasomnifera)

Ashwagandha is one of the most important herbs in the 3,000-year-old Ayurvedic Hindu system of medicine. Its name literally means "smell of horse" since the root does smell a little horsey. Its main use now is as a stress-relieving adaptogen. It has a significant effect on the

stress hormone cortisol, reducing it by 25%. It is very helpful at calming anxiety of all kinds, especially agoraphobia (fear of open places).

3. BACOPA (Bacopamonnieri)

Bacopamonnieri, sometimes called brahmi (from the word "brahman" meaning "the energy of universal consciousness"), is another important adaptogenic herb in Ayurvedic medicine. Bacopa has a long history of use for increasing longevity and enhancing brain power.

It reduces anxiety while also improving memory and attention. This makes it an excellent choice for anyone who has anxiety with memory loss.

4. GINKGO (Ginkgo biloba)

Ginkgo biloba is one the world's most ancient and impressive plants. The earliest ginkgo fossils date back 270 million years. Ginkgo trees literally grew when dinosaurs roamed the earth! A handful of ginkgo trees were at the epicenter of the Hiroshima atomic bomb blast. Amazingly, theses trees are so hardy that they survived and are still alive today. Ginkgo leaves have been used in traditional Chinese medicine for thousands of years.

While it's thought of as mainly a memory enhancer, ginkgo reduces the release of stress hormone cortisol making it effective for anxiety and stress as well. Ginkgo raises levels of the neurotransmitters serotonin and dopamine which are essential for a positive mood.

5. GINSENG (Panax ginseng)

Ginseng is one of the most ancient, popular and widely studied herbs on the planet. The Chinese believe it to be the "elixir of life" and have used it to promote strength, stamina, and physical performance for over 5,000 years.

It is usually labeled Asian, Chinese, or Korean ginseng, depending on where it is grown. Ginseng creates a relaxed, but alert, state. It calms you down and boosts your energy without being over-stimulating. It's useful for treating stress, anxiety, fatigue, and depression.

6. KAVA (Piper methysticum)

Kava (or kava kava) is a medicinal plant that originates in the South Pacific and is one of the most potent natural remedies for anxiety known. People throughout this region make a traditional kava tea that's valued for its ability to induce a state of relaxation and mental clarity.

Numerous studies consistently find kava effective at treating anxiety. It's been found to work as well as prescription medications for generalized anxiety disorder (GAD) by increasing the level of the brain chemical GABA (gamma-aminobutyric acid).

GABA is a relaxing neurotransmitter that calms the mind and puts the brakes on brain activity when needed. Low GABA is associated with numerous mental and physical disorders including generalized anxiety disorder, panic attacks, irritable bowel syndrome (IBS), and fibromyalgia. Kava should not be taken with many drugs and does not mix well with other natural remedies that can cause drowsiness such as 5-HTP, melatonin, gotu kola, valerian, and St. John's wort.

7. PASSION FLOWER (Passifloraincarnata)

Passion flower is the only herbal remedy on this list native to North America. This beautiful flowering vine was used traditionally by Native Americans to treat anxiety and insomnia. Studies have found it to be as good for treating generalized anxiety disorder as the prescription sedative Serax (oxazepam). It's believed to work by increasing brain levels of GABA.

8. VALERIAN (Valeriana officinalis)

Valerian has been called "nature's Valium" and is mainly used to relieve anxiety, stress, and insomnia. It is documented use in Europe goes back more than 2,000 years its properties were first described by Hippocrates.

It's another one of the several herbs on this list thought to work by increasing GABA levels in the brain. It is generally considered safe but unlike some of the other herbs for anxiety, valerian can definitely make you drowsy, so use it just before bedtime.Valerian can be consumed a tea or as a supplement, but most people stick with the supplement since valerian tea tastes and smells pretty awful.

1.13 NON PHARMACOLOGICAL TREATMENT FOR ANXIETY[100]

Anxiety disorders can be distressing and may often need therapy. The most important part of treatment of this condition is patient education. The guidelines especially for panic disorders, where patient may suffer sudden attacks without warning, recommend education for the family as well. The symptoms of an anxiety attack may appear similar to a heart attack or other medical ailments.

Patients as well the family needs to be educated regarding these symptoms. If a medical condition like high blood pressure, irregular heart rates and rhythms or overactive thyroid is found then appropriate medical therapy may be needed. Therapy includes psychotherapy and management with medications. The aim of therapy is to ensure that the patient functions adequately in their day-to-day life.

1.13.1 PSYCHOTHERAPY FOR ANXIETY

Psychotherapy includes cognitive-behavioral therapy, anxiety management therapy and applied relaxation therapy.

1.13.2 COGNITIVE-BEHAVIORAL THERAPY

Cognitive-behavioral therapy (CBT) is provided by a psychotherapist. Patient needs to be committed to therapy. Patients treated with a combination of CBT and medicines have better response than those who are undergoing usual treatment. Some 10 to 20 visits to the therapist are needed over a few weeks. For panic attacks 12 - 16 sessions over 3 - 4 months may be needed. These focuses towards recreating fear symptoms and help patients change their response to them.

In OCD for children CBT is the first choice of therapy. The therapy teaches the patient to identify and handle stress factors better. Patients will learn to decrease the sense of helplessness by shutting out or neutralizing panic-causing thoughts. He or she will be taught to avoid speculating that minor worries may turn to bigger problems. Relaxation techniques will be taught. For OCD the techniques are exposure and response prevention (ERP).

For patients with PTSD the psychological treatment will focus on the trauma with exposure therapy, cognitive therapy, and eye movement desensitization and reprocessing. Patient will be advised to lead a healthier lifestyle with regular exercise, adequate rest and sleep and

healthy balanced nutrition. They will be taught to avoid excessive caffeine and illicit drugs, alcohol and cigarettes. Family and social interactions to lessen the impact of the condition will be advised.

1.13.3 ANXIETY MANAGEMENT THERAPY

Anxiety management therapy involves education, relaxation training, and exposure to anxiety-provoking stimuli. However, there is no positive reconstruction technique to fight anxiety.

1.13.4 APPLIED RELAXATION THERAPY

Applied relaxation therapy helps patient relax each part of the body. The therapy takes 12 to 15 hour-long sessions in a number of sittings and has been found to be effective.

1.14 PSYCHOLOGICAL AND PHYSICAL STRESS MODELS [101]

Essentially, these models induce stress by exposing the animals to psychological or physical challenges. These procedures may be used in acute or chronic studies depending on the objectives and parameter chosen by the experimenter to evaluate the impact of stress on anxiety.

The main Stress protocols used are briefly described below.

1. Psychosocial stress

    a. Neonatal isolation

    b. Noise stress

    c. Circadian rhythm changes

    d. Predator stress

2. Physical stress

    a. Restraint stress

    b. Immobilization stress

    c. Temperature variation stress

d. Electric foot shock stress

1.14.1 NEONATAL ISOLATION STRESS

Early-life stressful experiences, such as maternal separation or neonatal isolation, promote long-lasting neural and behavioral effects and have profound consequences on subsequent quality of life. [102] During the neonatal separation procedure, on the 2nd day after birth, the litter of the inbred strain is removed from the cage and placed in another cage for 1 hour (9 a.m./12 a.m.) in a room located apart from the animal facility. White noise is played in the background to mask the vocalizations of other pups. After the 1-hour period, the litters are placed back with their dams in their home cages. The separation procedure is repeated for 8 days. This model has been used extensively to demonstrate the effect of early lifetime stress on vulnerability to addiction and in the generation of anxiety-like behaviors, which are usually observed in the adult rodents subjected to the contextual fear conditioning, EPM, or social interaction tests.

1.14.2 STRESS INDUCED BY CIRCADIAN RHYTHM CHANGES

Alterations in circadian rhythm have a profound impact on the physical and psychological homeostasis of an individual. Rodents subjected to unexpected changes in the day-night light cycle exhibit acute stress responses. Circadian rhythms are controlled by the pineal gland via melatonin secretion. The stress procedure consists of lighting the home cage of the rodents during the dark phase of the cycle (e.g., lights on from 7 p.m. to 7 a.m.) and leaving it unlit in the light phase (lights off from 7 a.m. to 7 p.m.). Another possibility is to promote four or five cycles of dark-light phases (60-180 minutes) during the circadian cycle. This is a good method for induction of short-term stress responses, but repeated exposure may lead to adaptation. Responses to this stressor can be evaluated by measuring biochemical parameters associated with stress response and using the previously described animal models of anxiety.

1.14.3 STRESS INDUCED BY A NOISY STIMULUS

Humans are constantly exposed to potentially hazardous levels of noise in modern daily life. In model animals, noise stress can be induced by using loudspeakers (15 W) connected to a white noise generator (0-26 kHz) located 30 cm above the cage. The noise can be set at a certain level (e.g., 100 dB or higher) and the animals can be exposed to the noise protocol either acutely or repeatedly (4 hours/day/15 days). Like those of other protocols, the behavioral effects of noise stress can be observed in animal models of anxiety and depression.

1.14.4 LOW TEMPERATURE-INDUCED STRESS

Changes in body temperature lead to stressful responses due to activation of the thermoregulatory center and, subsequently, of the HPA axis. Abrupt reductions in temperature by using either cold water or freezer compartments have frequently been used to induce stress in laboratory animals. The most widely used protocols consist in the immersion of the animals in cold water (15-18°C for 15-30 min) or placing the animals (in their home cages) in a cold, isolated environment (4°C for 15-30 min). This procedure can be used in acute or chronic protocols (7-14 days).

1.14.5 RESTRAINT AND IMMOBILIZATION STRESS

Restraint stress and immobilization protocols are one of the most commonly employed procedures to induce stress-related behavioral, biochemical and physiological changes in laboratory animals. Restraint stress is generally induced by keeping the animals in a cylindrical or semi-cylindrical tube with ventilation holes for 120-180 min. In an immobilization stress protocol, animals are restrained by gentle wrapping of their upper and lower limbs with adhesive tape for 120 min. Head movement is restricted by a metal loop wound around the neck. The procedure can be used to induce either acute or chronic stress (7-21 days). Immobilization models produce an inescapable physical and mental stress with a low rate of adaptation. After restraint or immobilization stress, animals exhibit higher levels of anxiety in the EPM and other tests of anxiety.

1.14.6 ELECTRIC FOOT SHOCK-INDUCED STRESS

This protocol is very similar to the pre-test session described in fear conditioning-based models. Rodents are very susceptible to mild shocks, exhibiting a remarkable stress response after foot shock delivery. The protocol consists of placing rodents in a chamber with a metal grid floor connect to a shock generator. After a habituation period, animals receive mild (05-2 mA), brief (1-2 s duration) foot shocks. Like other stress protocols, electric foot shocks can be combined with anxiety tests.

1.14.7 SOCIAL DEFEAT STRESS

The social defeat stress (SDS) model was initially proposed by Klaus Miczec. The SDS protocol consists of the introduction of a single mouse (known as the intruder) in the home cage of a resident male mouse (known as the aggressor). During the test, behaviors related to

confrontation of the intruder mouse by the resident aggressor are recorded. The time spent by an intruder mouse in social defeat posture induced by the presence of an aggressor is computed throughout five trials by a blind observer. Defeat posture is identified by the followed criteria: immobility (four paws on ground, oriented toward the aggressor), escape (escaping from the aggressor), crouching (four paws on ground, not oriented toward aggressor), or defensive upright stance (standing erect with forepaws extended). The procedure can be used in acute or chronic stress protocols.

1.14.8 CHRONIC UNPREDICTABLE STRESS

The chronic unpredictable stress (CUS) model has been widely used to induce persisting stress-related behavioral changes in rodents. It consists of randomly presenting different stressors to the rodents on a daily basis. This scheme prevents the stress adaptation process observed in other models of chronic stress. In this model, animals are exposed for 2 to 5 weeks to a wide range of stressors, including foot shocks, restraint stress, light-dark cycle reversal, unpleasant noises, changes in the home cage (removal of sawdust, replacement of sawdust with water, heating [37°C] or cooling [4°C] of the home cage). After several days of exposure to this regimen, the animals exhibit a gradually increased HPA axis sensitivity and a decrease in responses to pleasant stimuli, without; however, any change in exploratory activity.

2. LITERATURE REVIEW

2.1 LITERATURE REVIEW OF ANTI-ANXIETY

1. Anti-anxiety activity of Coriandrum sativum assessed using different experimental anxiety models

Poonam Mahendra *et al* evaluated that extract of C. sativum at 100 and 200 mg/kg dose produced anti-anxiety effects almost similar to diazepam, and at 50 mg/kg dose did not produce anti-anxiety activity on any of the paradigm used.

Indian J Pharmacol. 2011 Sep-Oct; 43(5): 574–577.

2. Anti-Anxiety Activity Studies of Various Extracts of Turnera aphrodisiacaWard

Kumar S *et al* investigated, petroleum ether (60–80°C), chloroform, methanol, and water extracts of T. aphrodisiaca aerial parts were evaluated for anti-anxiety activity in mice using elevated plus-maze apparatus. Among all the extracts, only methanol exhibited significant anti-anxiety activity at a dose of 25 mg/kg with respect to control as well as standard (diazepam, 2 mg/kg). The bioactive methanol extract was shaken with petroleum ether, chloroform, and n-butyl alcohol, and all the shakings as well as the remaining methanol extract (RME) were further evaluated for anxiolytic activity. Butanol fraction and RME were found to exhibit anxiolytic activity in mice at the dose of 10 mg/kg and 75 mg/kg, respectively.

Journal of Herbal Pharmacotherapy Volume 5, 2005 - Issue 4 Pages 13-21

3. Anti-anxiety studies on extracts of Passiflora incarnata Linneaus

Dhawan K *et al* evaluated that a fraction derived from the methanol extract of Passiflora incarnate has been observed to exhibit significant anxiolytic activity at a dose of 10 mg/kg in mice using elevated plus-maze model of anxiety. This fraction comprises mainly two components which are visible as blue and turquoise colored fluorescent spots at 366 nm of the UV light. The possibility of a phytoconstituent having benzoflavone nucleus as the basic moiety being responsible for the bioactivity of P. incarnata is highly anticipated.

Journal of Ethnopharmacology Volume 78, Issues 2–3, December 2001, Pages 165-170

4. Cross-National Study of the Extent of Anti-Anxiety/Sedative Drug Use

Mitchell B. Balter *et al* studied that in almost every country the percentage of females who had used anti-anxiety/sedative drugs was approximately twice that of males. Persons 45 years of age and over were over-represented among drug users in all countries in relation to their presence in the national population. The rank order of the countries on attitude toward tranquilizers was poorly correlated with rank order on use rates. However, within each country there was a sharp difference in attitude between users and nonusers. Independent data place the United States in a middle position among the nine countries surveyed on use of anti-anxiety/sedative drugs.

The New England Journal of Medicine 1974; 290:769-774

5. Increase of "antianxiety" activity and tolerance of behavioral depression during chronic administration of oxazepam

D. L. Margules *et al* evaluated that Oxazepam has two opposing actions on behavior: a response decreasing or depressant action and a response-increasing or disinhibitory action. The course of the two actions in chronic dosing was determined in rats in a test in which punished and unpunished schedules of reinforcement were alternated. The depressant action (measured by a decrease in the rate of unpunished behavior) was observed to undergo tolerance after 3–4 doses, while the disinhibitory action (measured by an increase in the rate of punished behavior) failed to show tolerance and even increased throughout the chronic series. The selective tolerance of the depressant action is probably due to neuronal adaptation, but changes in metabolism also may be involved. The increase in the rate of punished behavior is attributed, at least in part, to a progressive unmasking of the disinhibitory action as tolerance to the depressive action develops.

Psychopharmacologia, January 1968, Volume 13, Issue 1, pp 74–80

6. Anti-anxiety Activity of Citrus paradisi var. star ruby Extracts

Vikas Gupta *et al* reported that methanol extract at the dose of 100mg/kg of the leaves of Citrus paradisi var. star ruby markedly increased the average time spent in the open arms of the EPM. This effect was comparable to the effect produced by diazepam. Hence this plant may be developed as a potentially useful anti anxiety agent.

International Journal of PharmTech Research Vol.2, No.3, pp 1655-1657, July-Sept 2010

7. Anti-anxiety effects of progesterone and some of its reduced metabolites: an evaluation using the burying behavior test

O.Picazo *et al* studied that the 3α-5α metabolite of progesterone shows the highest anxiolytic potency in the burying behavior test, when compared with all steroids evaluated. The data are discussed in terms of the close structure-activity relationship requirements of steroids to stimulate the GABA/benzodiazepine receptor-chloride ionophore complex.

Brain Research Volume 680, Issues 1–2, 22 May 1995, Pages 135-141

8. Thigmotaxis as a test for anxiolytic activity in rats

Dallas Treit *et al* suggested that when effects on general activity were factored out using analysis of covariance, the test also showed some degree of drug-class specificity, since neither d-amphetamine, morphine, nor chlorpromazine produced this anti-thigmotaxic effect. These results support an earlier report that thigmotaxis may be a useful test for anxiolytic activity in rats.

Pharmacology Biochemistry and Behavior Volume 31, Issue 4, December 1988, Pages 959-962

9. Behavioural Validation of a Light/Dark Choice Procedure for Testing Anti-Anxiety Agents

Rend Misslin *et al* investigated about the present results indicates that this procedure can be considered as an unconditioned conflict paradigm based at once on the innate tendency of mice to seek refuge in a dark box and their propensity to escape novel places into which they have been forced. It is suggested that this test is particularly well adopted to measuring the antiaversive properties of the so called “anxiolytic” drugs.

Behavioural Processes Volume 18, Issues 1–3, 1989, Pages 119-132

10. Anti-anxiety effects of Apocynum venetum L. in the elevated plus maze test

Oliver Grundmann *et al* evaluated that ethanolic extract prepared from the leaves of Apocynum venetum (AV) using the elevated plus maze (EPM) in mice results that extract is an effective anxiolytic agent, and suggest that the anxiolytic-like activities of this plant are mainly mediated via the GABAergic system.

Journal of Ethnopharmacology Volume 110, Issue 3, 4 April 2007, Pages 406-411

2.2 LITERATURE REVIEW ON VACHA CHURNA

1. Effect of Brahmyadi Churna (Brahmi, Shankhapushpi, Jatamansi, Jyotishmati, Vacha, Ashwagandha) and tablet Shilajatu in essential hypertension: An observational study

Arshiya Ali *et al* studied essential hypertension with Brahmyadi Churna and tablet Shilajatu for a period of 1 month with milk as Anupana. Observation was done before the treatment, 3 mid test assessments on 7th, 14th, and 21st day post test assessment was done on 30th day. Intervention revealed that 19 had marked improvement, 14 had moderate improvement, 5 had mild improvement, and no improvement was noticed in 2 individuals. Reduction in blood pressure was observed markedly with $P < 0.000$.

J Adv Pharm Technol Res. 2015 Oct-Dec; 6(4): 148–153.

2. RATIONALITY OF SWARNA PRASHAN IN PEDIATRIC PRACTICE

Dr. Mahapatra Arun Kumar *et al* in this samskara, swarna Bhasma with herbs like Vacha Churna (Acorus calamus), Brahmi (Bacopa Monnieri) mixed with Honey and ghee is administered to the new born baby for enhancing immunity as well as for intellectual development. All ancient Ayurvedic texts and particularly Kashyap samhita, details of swarna lehan with it therapeutic utility is described. Swarna Prashan is done by various means including raw gold, Swarna Bhasma etc. Studies shows that by classical bhasmikaran process as described in texts of Ayurveda, there is reduction in the particle size of gold to dimension of about 56-57 nm. Analysis of Various experimental studies shows that Swarna Bhasma possesses immunomodulatory, free radical scavenging activity, antistress activity and analgesic activity. Toxicity study shows that chronic administration of Swarna Bhasma is non toxic as judged by various laboratory and histological parameters. However, scientific evidences regarding the safety and efficacy of Swarna prashan in pediatric practice is lacking and hence this practice should be avoided or used with utmost caution.

International Journal of Ayurvedic and Herbal Medicine 3:3 (2013)1191:1200

3. A comparative acute toxicity evaluation of raw and classically processed rhizomes of Vacha (Acorus calamus Linn.)

Bhat, Savitha D *et al* reported that at 2000 mg/kg dose both raw and classically processed Vacha did not produce any observable toxic effects and all animals survived 14 days of observation.

IJNPR Vol. 3(4) [December 2012], 506-511

4. A Recent Approach for Development and Standardization of Ayurvedic Polyherbal Formulation (Churna) for Antioxidant Activity

S. Ghosh D *et al* reported that the Physicochemical constituents found to be present in the raw material used the preparation of Antioxidant churna possible facilitate the desirable therapeutic efficacy of the medicinal formulation, and also could help in knowing the underlying mechanisms of Pharmacological Action.

American Research Journal of Pharmacy, Volume 1, Issue1, Feb-2015

5. ANTIEPILEPTIC AYURVEDIC HERBAL DRUGS – A REVIEW

Janani *et al* reported that the ayurvedic herbs which are having lesser side effects in comparison to synthetic drugs can be a better option for the control and treatment of epilepsy .The herbs like Vacha, Sankhapuspi, Tagar, Shatavari, Kushtha etc. have been mentioned in the Ayuvedic classics for the management of Apasmara and also experimentally proved as anticonvulsant.

Pharma Science Monitor 6(4), Oct-Dec 2015

3. AYURVEDA[103]

Disease is the disturbance of ease i.e. comforts freedom from constraint, annoyance, awkwardness, pain or trouble both bodily and mental. Since time immemorial, man has tried to lead a disease free life. In one of the oldest repositories of human knowledge, vedas of Aryans (veda means to know, knowledge), the plants with medicinal virtues have been identified as oushidhis which are to be distinguished from ahara the edible plants. After vedas, further developments in various spheres of human life gave rise to Indian medicine called Ayurveda (Ayuh – life, veda – science). Dhanwantri, said to be the father of Indian medicine, lived in 7th century BC., as compared to Hippocrates who lived in 5th century BC. After Dhanwantri, the system of Ayurveda developed further, and peaked at the time of CharakSanhita and SushrutSanhita, the treatises on medicine and surgery written about 1000 BC to 1000 AD. CharakSanhita is said to be a compilation of proceedings of a symposium held in the Himalayas, to discuss the cure of various diseases which had originated at that time due to urbanization.

For this purpose, the ancient scholar divided Ayurveda into the following eight disciplines:

1) Internal medicine
2) Ophthalmology and otorhinolaryngology
3) Surgery
4) Toxicology
5) Psychiatry
6) Paediatrics
7) Rasayana, which in broad terms means rejuvenator
8) Vajikarna, the literal meaning is to have the sex power of a horse, but is often considered as fertility inducer or procreator.

Ayurveda, the Ancient Science of Hindus and Indians, dates back about 7000 years. It has eight branches, one of which is Rasayana Tantra. The word rasayana literally means the path that rasa takes (rasa: the primordial tissue or plasma; ayana: path) (Charaka). It is also considered as the science which restores youth, alleviates suffering (diseases) and bestows longevity (Sushruta). It is believed in Ayurveda, that the qualities of the rasa-dhatu influence the health of other dhatus (tissues) of the body. Hence, any medicine that improves the quality of rasa are called as rasayanas, resulting in the strengthening or promoting of the qualities and health of all tissues of the body. These rasayana plants are said to possess the following properties:

- ❖ Prevent ageing
- ❖ Re-establish youth
- ❖ Strengthen life
- ❖ Strengthen brain and mind
- ❖ Prevent disease
- ❖ Promote healthy longevity

Rasayana Tantra appears to have been practiced as an independent clinical discipline primarily as a positive health medicine. With the passage of time this important branch of knowledge has ceased to be in practice (except the knowledge and use of a few herbs) in its appropriate form. Comprehensive efforts are needed to revive this useful discipline for the welfare of humanity at large.

The ability to adapt to a given habitat is a distinctive feature of all living organisms. Any type of demand (external or internal) in this habitat elicits either a specific or non-specific response.[104]This non-specific response to any demand has been defined as stress and the demand as stressor by Hans Selye. As per his description there are three stages of response to any given situation of stress, which together constitute the general adaptation syndrome (GAS) [105], the stages being

a) alarm reaction
b) resistance
c) Exhaustion.

Thus, the ability to develop resistance and to maintain it is crucial for coping with a variety of stressors encountered in human life. The desire to control the coping mechanisms has led to the origin of the science of adaptation. The branch of Ayurveda which deals with the science of adaptation is called Rasayana Tantra. Ayurveda may not coin the term adaptogen, but those practices, or substances / herbs / medicines which help a person cope with his day to day stresses are known in Ayurveda as Rasayanas. Modern research has proved that herbs listed as Rasayanas possessadaptogenic properties as well. All of the above imply that they improve and increase the resistance of the body. The scientific studies carried out on most of the rasayana herbs showed that theseplants non-specifically activated the reticuloendothelial system (RES) and other components of the immune system as well. [106]

Knowing that the central nervous system, endocrine system and the immune system participate in intense crosstalk[107], it was easy to hypothesize that by acting on the immune system these rasayanas could exert broad-based effects by initiating a massive cascade of events involving various neurotransmitters, hormones and amines of the stress response cycle.

With the emerging science of ecogenetics, it is becoming evident that genetic predisposition can alter responses to the external environment. However, at present most of the data is on diseases, which manifest in genetically predisposed persons on exposure to the external stressors. Can rasayanas (or adaptogens or immunomodulators) influence genetic control and favour the maintenance of homeostasis in stressful situations? The research on Amruta (Tinosporacordifolia) opens up a new vision in this direction, as early experiments have shown that it increases the bone marrow proliferative fractions leading to leucocytosis. A slight increase in dose reverses this process and inducing apoptosis, and this apparent paradox was believed to be because of its effect on c-myc, a gene that causes both proliferation as well as induces apoptosis depending on the environment.

These are some of the recent advances seen in the field of rasayana drugs. Looking at it appears that rasayana herbs act as:

- an adaptogen
- an immunostimulant
- an immunomodulater
- pro-host probiotic
- anti-mutagenic

But Ayurveda has much more in it to be included under rasayana therapy. Looking at only a few herbs will be doing an injustice to the holistic approach of Ayurveda, hence a brief description of Rasayana Tantra may be necessary here.

According to Ayurvedic concept Rasayana therapy simultaneously affects the body and the mind and brings about physical and psychic improvement. This therapy prevents the effects of early ageing, develops intelligence and increases the bodyresistance against diseases.

Rasayana means vitalizing / rejuvenating. In the words of Charaka with a rasayana, one obtains longevity, regains youth, vitality and vigour, gets a sharp memory, intellect and freedom from diseases, gets a lustrous complexion and the strength of a horse. Sushruta is more specific,

describing a rasayana as one which is anti-ageing; increases life-span promotes intelligence and memory and increases resistance to disease.

Any drug, diet or conduct that leads to the replenishment of the dhatus and enlivens the body and mind is rasayana. Rasayana not only rejuvenates the body and mind, it also prevents disease. There are a number of drugs/materials described which possess the qualities of maintaining health, prolonging life and warding off diseases. They are all grouped as rasayana in Ayurveda. A close look at the concept of rasayana of Ayurveda and various research findings on rasayana suggests that rasayanatherapy/ drugmay have its effect on our ojas,immunity,resistance, etc.

Hence the following points related to the concept of resistance, immunity as described in Ayurveda may be useful:

1.The qualitative, quantitative and functional balance of the body-elements maintains strength which, in general, causes resistance to diseases. Raktam (Blood) has been attached much importance because it's normal condition reflects good health and general resistance power.
2. Ayurveda has described a separate substance named ojas which has been said to be the essence of all the dhatusand is considered to be an excellent body element. Therefore, the excellence of the body in totality is ojas. It reflects the excellent performance of the man as a whole. Therefore, resistance power of the body depends on the quality and quantity of ojas.
3. Bala (strength or power) has been classified as sahaja (natural, hereditary), kalakrita (variable as per age and season) and yuktikrita (acquired by good diet, drug and exercise etc.). This is why some families have a specific resistance to specific diseases; some diseases are born in a specific age and season and generally speaking those, who are well built, fall less ill.
4. Again the bala (resistance) has been divided into vyadhi pratibandha katwa and vyadhi bala virodhitwabala. The former is the specific resistance against a specific disease so that those diseases will never afflict the man. Today, vaccines substitute this type of resistance. The latter does not stop the onset of the disease but can only minimise its severity. In another context it has been said that the overweight or emaciated, those of weak build, who have a deficient diet and who are mentally weak, are more susceptible to disease.

There are various ways of classifying rasayana therapy in Ayurveda. They are basically either based on the method of administering the rasayana therapy, such as

- kutipravesika (indoor)

- Vataatapika (outdoor)
- Kaamya (invigorating and vitalizing)
- Medhya (promoting intellectual factors)
- Achara (address psycho-somatic activities)
- Naimittika (used to promote resistance against disease following illness)
- Vrishya (virilifying or sex-stimulant).

Also remember that Rasayana Tantra is one of the eight branches (Asthanga Ayurveda) of Ayurveda. And, before administering rasayana the individual needs to undergo the shodana/panchakarma(purification therapy) process. Kaya-kalpa is nothing but another name for rasayana.

3.1 WHAT ARE RASAYANA

The agents which cure body and mental diseases, delay old age, increase mental power, generating power, vital energy, eyesight, impart intelligence, memory, aid proper digestion and clear complexion are Rasayana.[108] These nourish the whole body by strengthening the primordial tissue Rasa, the essence of all food we take, and which the body can assimilate. If this essence is well distributed in all systems, the body remains healthy. By their physio-chemical action

Rasayana purify and promote dhatus (tissue). They augment the body's disease resistance capacity, as well as the ability for restorative reaction and counteract all the deleterious effects including that of ageing.

The Rasayana keep tissues, enzymes, membranes and tranquility of the mind in their normal functioning conditions through anabolic processes. They help if the tissues have become inactive, are to be revitalized and their composition changed. They achieve this by increasing bala, the physiological and immunological strength of the body. They are good for all people, at all stages, at all times but they do not prevent ageing and do not assure immortality.

Rasayana may be compared to alteratives, which work as blood cleansers by their diuretic and antihepatotoxic action. Alteratives also restore the proper functions of the body and increase health and vitality. [109]They alter the body's metabolism in various ways so that the tissue can best deal with the range of function from nutrition to elimination. Some of these herbs eliminate waste from the body through kidney, liver, lung or skin, while others work by stimulating digestive functions and still others act as antimicrobial agents. Some Rasayana also help in the disposal of waste ama. Agni is the biological fire burning inside the body, which acts

on food so that energy is generated by digestion. If waste is not excreted from the body properly and ama is deposited in the tissues, it may disturb various systems. The herbs are given to strengthen the tissue to counteract all the ill effects of ama.

In Ayurveda, human life has been divided into four spans according to growth and development:

1) From birth to the age of 20, all tissues of the body grow.
2) 20–40 years of age, tissues continue to grow.
3) 40–60 years is the age of stagnation. If an individual has a balanced diet, cheerful life, adequate nourishment, and is free of worries and anxiety, the person can maintain good health. At this stage the mental activities expand, and power of judgment increases.
4) After the age of 60, in spite of good quality of life, senescence starts. Metabolism of the tissues decreases, waste is not excreted properly, and the bone joints become dry and fixed.

The body can be rejuvenated at stages 3 and 4 and during senescence by Rasayana. These can be used as a dietary supplement, but in the case of chronic diseases, old age, etc. where kaya kalp (rejuvenation) is required, a special treatment, called Rasayana therapy, is provided under the supervision of medical experts.

3.2 RASAYANA THERAPY

Before the start of actual therapy, the internal and external organs of the body are cleansed and the system is made more receptive to assimilation of medication. It has been stressed that Rasayana administered without these treatments is like seed sown on barren land, from where no good results can be expected. This pretreatment is given only to those persons who have enough strength to bear it.

3.3 PRE-TREATMENT FOR RASAYANATHERAPY

3.3.1 SAMSHODHNA (Diet restriction)

One week before the start of therapy, the person should resort to a simple diet consisting of only steamed vegetables and fresh fruits. He should not be given sugar, alcohol or animal products. Milk and honey are allowed.

3.3.2 PURVAKARMA (Preparatory treatment)

Involves massage with warm oil, and application of heat on the body by a massage therapist. The massage should be so vigorous that a large amount of latent heat of the body is generated. For this purpose sesame oil is generally used but sometimes some other medicated oils, which are supposed to have better properties, are applied.

3.3.3 PANCHKARMA (Cleansing of internal organs of the body)

Virechna : purging to clean the small intestine

Basti : to clean the large intestine

Vamna : emetics to clean the stomach

Naysya : nasal drops to clear the respiratory passage

Raktaoksha : Bloodletting by leeches may be done to those patients who have excess of blood in their body. Nowadays blood is donated to the blood bank. It is optional.

3.4 ADMINISTRATION OF HERBS

After panchkarma the patient is admitted into a hut (kutiprveshika) for the administration of Rasayana preparations. The hut should be in a pollution-free area, well ventilated, facing north, and painted with slaked lime to make it germ-free.

The preparations required during Rasayana therapy vary, at the discretion of the health provider. Generally the patient is given a restricted diet, along with only that quantity of medication which the patient can easily digest. The medicine should be fully utilized by the body and not passed into faeces undigested. The medicine should not create any digestive disturbance, such as indigestion, hyperacidity or constipation. If any of these conditions persist for some time, a laxative or a colon cleanser should be given.

Herbs such as garlic, neem, amalaki, etc. were commonly used for this therapy but now polyherbal preparations with animal products such as musk, amber, coral, pearl, gems, minerals, and metals are prescribed. Some of the formulations may consist of red sulphide of mercury, prepared in the presence of gold, along with processed poisonous herbs like aconite, nux vomica, etc. The preparation is given to the patient in the early hours of the morning (within three hours of sunrise). During administration of mercurial compounds, the patient should have a pure

vegetarian diet. He should not remain thirsty or hungry. He should avoid excessive sleep, swimming, talking, anger, sadness, desire, excessive happiness, bathing, worry, irritation, depression, sex, and fragrances. He should lead a simple, pious, and religious life.

After kutiprevesh, the patient should start steadily with a diet of rice water, followed by rice gruel, rice and split beans, rice with beans, and finally he may be given a normal diet with a teaspoonful of ghee (butter oil). The patient should remain in a relaxed condition, and should have a whole body massage daily with warm sesame oil. This treatment is said to make the arteries softer and smoother, the constitution of the blood changes, clots are dissolved, and new cells are formed.

3.5 RASAYANA PREPARATIONS

The simplest mode of administering an herb is to take it as it is, to pound it or to use its juice after filtration. These are the least processed forms and all constituents of the herb are made available to the body. However fresh herbs are often not readily available throughout the year. Some fresh herbs not only taste bad but are even nauseating. The best alternative to fresh herbs is to use properly dried herbs in powder form (**churn**). Usually the churn is mixed with salt or sugar to make them more palatable. These powders are usually administered with a fluid medium, because in a dry form they are difficult to swallow or sometimes may even choke the respiratory passage. For Rasayana powders, the common medium for administration is boiled lukewarm cow's milk, sweetened with sugar, honey, cow's ghee or a mixture of both milk and ghee. (Ghee is prepared by heating the butter to remove the fat-insoluble, proteinaceous and other matters and water, so that only fatty acids and the constituents soluble in fat are left. It is surprising to find that though butter has been a common household item in India since ancient times, it has rarely been used for the administration of Ayurvedic preparations.)

The other methods of administration of powdered herbs are infusion, decoction and distillation. To make an infusion, the herb is immersed in water, usually kept overnight, filtered and used. In decoction the herb is boiled in water until the water is reduced to half or so, filtered through a cheese cloth and heated further until the decoction is reduced to one-fourth. The infusion or decoction may be used in place of juice, if fresh herbs are not available. For distillation, fresh leaves or flowers, dry root or seed are boiled in water in a closed container and the steam that arises is condensed, so that the volatile active constituents of the herb are dispersed in water (aqua).

Ghansatva are concentrated, sometimes standardized aqueous extracts of the herbs made from the decoction in the form of a thick paste or dry powder. These are now preferred by the pharmaceutical industry because they reduce the bulk of the herbs, and can be easily incorporated into capsules or pills, are easy to formulate, have a longer shelf life and the end products have good consumer appeal. Sometimes syrups or alcoholic preparations are made from these extracts.

The herbal products, in general, have an expiry period of one year but if stored in airtight containers they may be used for up to two years, whilst the mineral products may be used indefinitely. The early Ayurvedic physicians were aware that the aqueous decoctions did not extract all the constituents from the herbs, so they developed methods to generate alcohol from the solvent during fermentation, in which ethanol soluble constituents of the herbal mixtures could be dissolved. These preparations are called **Asav and Arishta**, to which sweetening agents such as raw sugar, honey and

Rasayana preparations flowers of Woodfordiafloribunda,etc., which can be fermented easily, are initially added. Due to the alcohol these preparations have a long shelf life, taste good and provide immediate energy and therapeutic agent to the body. Asava and Arishta differ in their method of preparation. Arishta are prepared from the decoctions of the herbs, whereas Asav are made from dry powders. In both cases all the herbs are allowed to ferment in airtight earthen pitchers for 40–50 days. Before the fermentation of herbal materials it is ensured that the pots are clean. They are then fumigated with camphor, sandal and agar wood (Aquillariaagallocha), or with long pepper powder and ghee. The water to be used should not be alkaline and should be free of inorganic matter. When the period of fermentation is complete, the solution is filtered and stored in bottles. The fermented end product mainly consists of ethanol, glycerol, lactic acid, acetic acid, besides other products. This can be used for an indefinite period. In a study of Draksharishta, it was seen that before fermentation the mixture contained 34.91 per cent sugar, but after fermentation the product had 19.17 per cent sugar, 8.70 per cent, alcohol, 0.24 per cent glycerin, 0.21 per cent lactic acid and 1.38 per cent acetic acid. The pH of the medium changed from 6.40 to 5.00.

Avleha or lehya (linctus) are semi-solid preparations prepared from dry powders, decoctions and the pulp of the herbs, along with sugary substances, honey, ghee and/or oil. During preparation, sugar is boiled in the extract/decoction of the herbs or water, until thick in consistency. A fine powder of herbs, minerals and spices is then added to warm thick syrup and

stirred so that a homogenous mixture is formed, followed by ghee or oil when still hot. Honey should be added after cooling the preparation. Avleha should be dried to such an extent that there is neither too little nor too much moisture.

The term **Bhasam** literally means ash. In practice, in this case, minerals, gems or hard animal products, after treatment with herbs, are calcined at a very high temperature to form ash and then this ash-like end product is heated and powdered repeatedly. When this process has been repeated 100 times, the bhasam is known as Shatputi (shat is hundred) but after 1,000 times it is known as Sahastraputi (sahastr is thousand). For each mineral or exoskeleton of animals, different methods are followed. These products do not degrade during storage and can be used indefinitely.

Ghrita or Ghritam are preparations made from oil or ghee. These contain fat soluble constituents. Ghrita are prepared by heating juice, paste, decoction, infusion, etc. of the herbs in ghee and oil until all the water evaporates. The resulting mass is filtered to remove fat insoluble materials. The fat is preserved for use as a medicine. Where vegetable oils alone are used, heating is stopped when froth appears, but when ghee is used, heating is stopped when the froth subsides. For internal uses these preparations can be stored for sixteen months after manufacture, but for external use there is no expiry period.

Pak are the preparations made from milk, ghee, dry fruits, herbs, minerals and spices. These preparations are mainly used as nutrients or aphrodisiacs. The usual method for their preparation is to boil milk until thick and solid. This condensed milk is fried in ghee and sugar powder or thick sugar syrup, herbs, minerals, chopped dry fruits and spices, etc. are added to it when warm. The whole mass is made into chocolate or candy balls of about 25 g each.

Before use poisonous herbs or minerals are subjected to mitigation called **marn** (to kill) or **sodhana** to purify but it is actually a process to reduce the toxicity so as to make them available to the body within safe therapeutic doses.

In this process toxic materials are generally boiled or treated with some herbs or chemicals in fluids such as cow's urine, cow dung solution, water, milk, etc. After treatment the herb/mineral is dried and processed further before use.

In India, cane sugar juice is used for making raw sugar (brownish in colour) and for sugar manufactured by western technology. In addition to these, an indigenous method of refining sugar has been used for a very long time. The end product, called Mishri, consists of

lumps of transparent, bold crystals. This sugar candy is considered to have a cooling effect and is used in auspicious ceremonies and in Ayurvedic medicaments as a sweetening agent. In the present book, the term sugar candy has been used for Mishri, to differentiate it from other forms of sugar.

In Ayurveda five types of salts are recognised. Out of these a man-made preparation called black salt is considered a good carminative and is included in many formulations for digestive problems. It is prepared by fusing saltpetre (nitre), Terminalia chebula, and common salt. In the presence of organic matter at high temperatures the salt turns into a deep violet amorphous mass.

4. VACHA[110]

Vacha is also called Calamus and Sweet Flag in English and Botanically, *AcorusCalamus*. is a potent nootropic herb.

Fig. 3: Acorus Calamus

4.1 BOTANICAL CLASSIFICATION

Kingdom	-	Plantae
Sub-Kingdom	-	Viridiplantae
Infra Kingdom	-	Streptophyta (Land Plants)
Super division	-	Embryophyta
Division	-	Tracheophyta (Tracheophytes or Vascular Plants)
Sub Division	-	Spermatophytina (Spermatophytes or Seed Plants)
Class	-	Magnoliopsida
Super Order	-	Lilianae (Monocots or Monocotyledons)
Order	-	Acorales

4.2 BOTANICAL DESCRIPTION

Vacha is the Hindi Name of Calamus root, also known as Sweet Flag in English. Botanically, it is Acorus Calamus, which belongs to the Acoraceae (sweet-flag).

Botanical Name	-	*Acorus Calamus*
English Name	-	Calamus, Sweet Flag
Hindi Name	-	Vacha, Vach
Ayurvedic Name	-	Vacha, Ugargandha, Chhadgrantha
Hindi	-	Bach, Gora-bach
Tamil	-	Vasambu, Pillai maruntho
Telugu	-	Vasa
Kannada	-	Baje, Narru Berua
Malayalam	-	Vayambu

4.3 THE PLANT AND ITS DISTRIBUTION

This herb originated in east Europe and adjoining areas. In India it grows in marshy and humid land in many places. It has sword-like leaves, arranged in a rosette, from the centre of which a bunch of blue or violet flowers may arise, hence the name blue flag. The root is about 1 cm thick, spreads prostrate within the soil and has distinct nodes and internodes. Numerous root fibers arise all over the internodes.

During collection, the root is removed from the soil, cut into 5 cm long pieces and dried. On drying it becomes rather flat with longitudinal striations. The central vascular portion is darker than the rest of the root. It has a distinct strong aromatic odour.

In Indian it is known as vachaor ghorvachaand it is distinguished from balvachaor parsikvacha (khurasanivacha) which does not have a central darker portion and is white throughout. It is obtained from Iris germanica L, which is cultivated in graveyards of Muslims in Kashmir. It is said that the root's smell keeps predators (rats) away, which would otherwise prey on the dead bodies.

4.4 DESCRIPTION

4.4.1 MACROSCOPIC

Drug occurs in simple or rarely with thumb-like branches at nodes; sub cylindrical to slightly flattened, somewhat tortuous or rarely straight, cut pieces of 1-5 cm long, and 0.5-1.5 cm thick; upper side marked with alternately arranged, large, broadly, triangular, transverse leaf scars which almost encircle the rhizome; at nodes leaf sheath mostly having an appearence present; lower side shows elevated tubercular spots of root scars; light-brown with reddish-tinge to pinkish externally, buff coloured internally; fracture, short; odour, aromatic; taste, pungent and bitter.

4.4.2 MICROSCOPIC

Rhizome - Shows single layered epidermis; cortex composed of spherical to oblong, thin-walled cells of various sizes, cells towards periphery, smaller, somewhat collenchymatous, more or less closely arranged cells towards inner side, rounded and form a network of chains of single row of cells, enclosing large air spaces, fibro-vascular bundles and secretory cells having light yellowish-brown contents, present in this region; endodermis distinct; stele composed of round, parenchymatous cells enclosing large air spaces similar to those of cortex and several concentric vascular bundles arranged in a ring towards endodermis, a few vascular bundles scattered in ground tissues; starch grains simple, spherical, measuring 3-6 μ in diameter, present in cortex and ground tissue.

Powder - Buff coloured; shows fibres, reticulate, annular vessels and simple spherical starch grains, measuring 3-6 μ in diameter.

4.5 PHARMACOLOGICAL ACTIONS

Vacha (*Acorus Calamus*) has a wide range of therapeutic uses. It has antispasmodic, carminative and digestive properties, which helps in the treatment of the abdominal diseases. It has stimulant action on the mind and increases attention span and concentration. Anti-inflammatory and analgesic actions help in arthritic disorders especially rheumatoid arthritis. Overall, it improves blood circulation and mitigates the inflammation.

4.6 MEDICINAL PROPERTIES

- ✓ Neuroprotective
- ✓ Anticonvulsant
- ✓ Antidepressant
- ✓ Nervine Stimulant
- ✓ Nootropic
- ✓ Sedative
- ✓ Muscle relaxant
- ✓ Analgesic
- ✓ Anti-arthritic
- ✓ Anti-inflammatory
- ✓ Antitussive
- ✓ Antibacterial
- ✓ Anti-hypertensive
- ✓ Cardiac Stimulant
- ✓ Cardio protective
- ✓ AamPachak (Detoxifier)
- ✓ Carminative
- ✓ Antispasmodic
- ✓ Cholagogue
- ✓ Digestive Stimulant
- ✓ Emmenagogue
- ✓ Intellect promoting
- ✓ Thermogenic (produces heat in the body)

4.7 AYURVEDIC PROPERTIES

RASA (Taste)	KATU (Pungent), TIKTA (Bitter)
ANU RASA (After Taste)	KASAYA (Astringent)
GUNA (Main Quality)	LAGHU (Light), TIKSHNA (Sharp)
VIRYA (Potency)	USHNA (Hot)
VIPAKA (Resultant)	KATU (Pungent)
PRABHAVA (Action)	Nootropic
DOSHA KARMA (Effect on Humors)	Pacifies KAPHA & VATA and increases PITTA
Dhatu (Tissue) Effect	RASA, RAKTA, MEDAS, ASTHI
Organs Effect	Brain, Vocal Cord, Stomach, Liver, Lungs, & Uterus
Main Indication	Skin Disorders

According to ayurvedic point of view, it is good for Vata and Kapha disorders and it should be avoided in Pitta dominance and increased Pitta symptoms.

4.8 VACHA (CALAMUS) INDICATIONS

When used as tonic, Vacha (Calamus) root helps to increase:-

- Memory
- Cognition
- Intelligence
- Confidence
- Digestive fire
- Lifespan

Vacha (Calamus) root is widely used for many ailments and some of them are listed below:

Mind, Brain & Nerves

- Memory Loss
- Mental Fatigue
- Headache
- Epilepsy

- Schizophrenia
- Anxiety
- Depression with passive symptoms
- Postpartum depression
- Paralysis
- Delayed speech and language milestones in children

Abdomen

- Diseases due to accumulated AMA (toxins from undigested foods or particles)
- Loss of appetite
- Loss of desire to eat
- Constipation (due to excess AMA)
- Flatulence
- Abdominal cramps
- Piles
- Worm infestation – intestinal worms
- Irritable bowel syndrome
- Bad breath
- Diarrhea

Respiratory System

- Productive cough
- Upper respiratory tract infection and fever due to it
- Hiccup
- Asthma
- Common cold or Flu

Muscles, Bones & Joints

- Fibromyalgia or muscle pain
- Frozen shoulder
- Rheumatoid arthritis

Heart & Blood Vessels

- Tachycardia

- Hypertension

Women's Health

- Dysmenorrhea
- Oligomenorrhea

4.9 SIDE EFFECTS

The most common side effect of Vacha (sweet flag) is **headache**, which generally occurs in people with increased Pitta Dosha in their body.

1. Headache (common in people with Pitta Constitution or having Pitta disease)
2. Nausea
3. Vomiting (occurs only with higher dosage i.e. more than 1 gram)
4. Low blood pressure (uncommon with Calamus root powder, but common with Calamus oil)
5. Bradycardia – slow heartbeat

5. SCOPE OF WORK

There are an estimated 40 million people above age 18 that suffer from anxiety, clearly more emphasis will be placed on coming up with treatments will be burden

There are currently many different types of medications that can be utilized to treat anxiety. The problem is that many of the drugs are poorly tolerated and/or produce significant unwanted side effects.

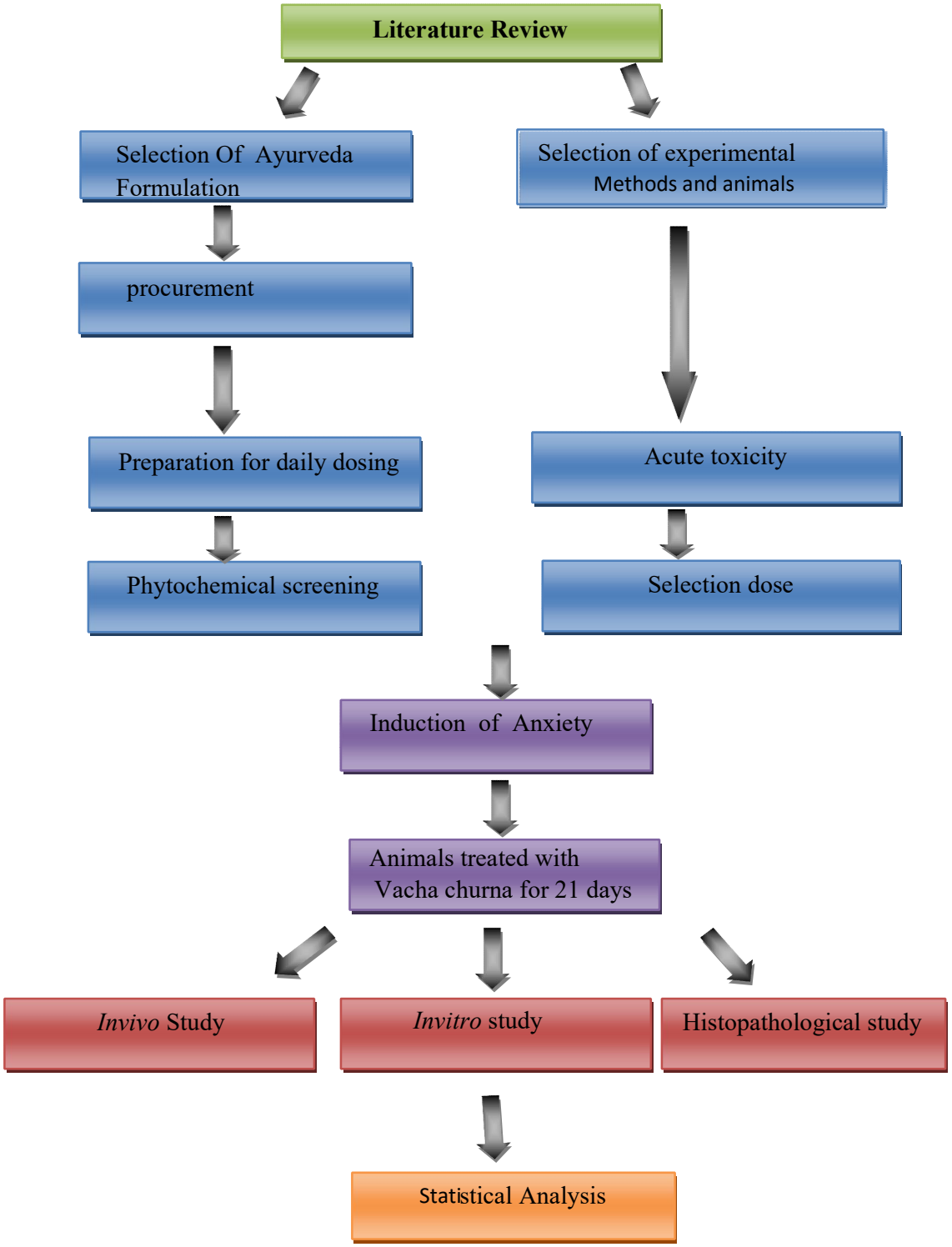
The most effective class of drugs to treat anxiety is the benzodiazepines as they provide rapid and significant relief but the problem with the benzodiazepines is that they impair cognitive function while consuming them, and long-term usage could lead to permanent memory impairment.

Most people end up preferring alternative treatment such as natural cures for anxiety like

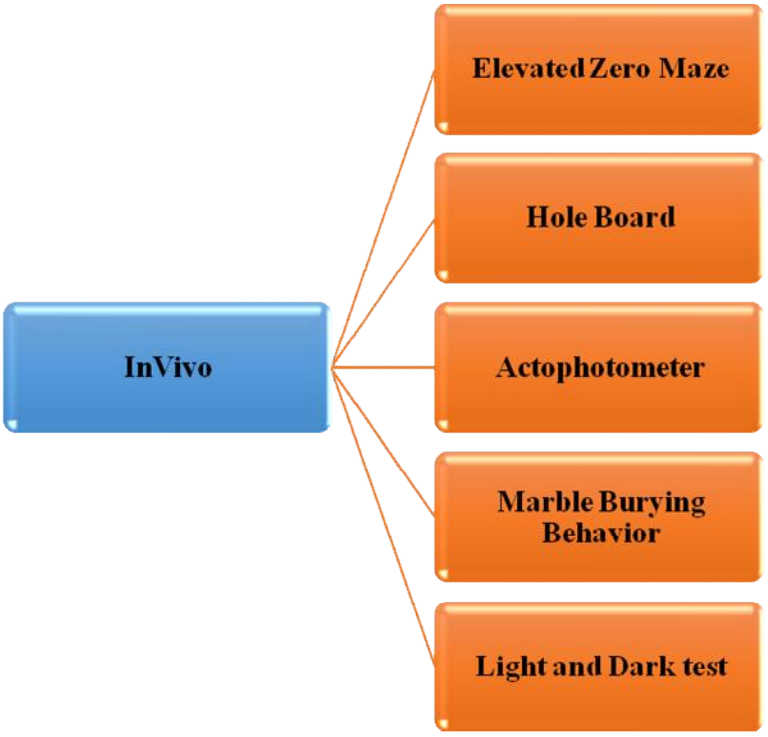
- Meditation practice such as Ayuveda, Siddha and Homeopathy
- Participating in cognitive behavioral therapy (CBT),
- Reducing stress
- Exercise.

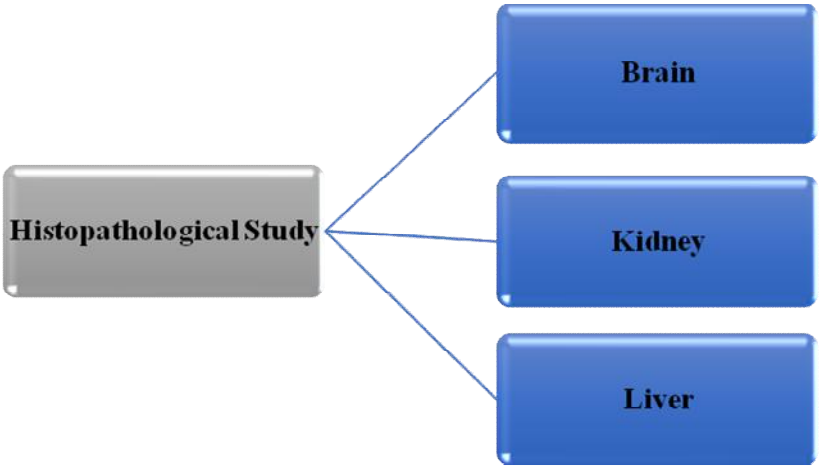
There are people that have explored every logical method of biohacking their mental health and have still not been able to find relief. Sometimes people may genuinely need a medication to help them cope with their reality. So an effort has been made to validate the effect of ayurvedic preparation Vacha Churnam

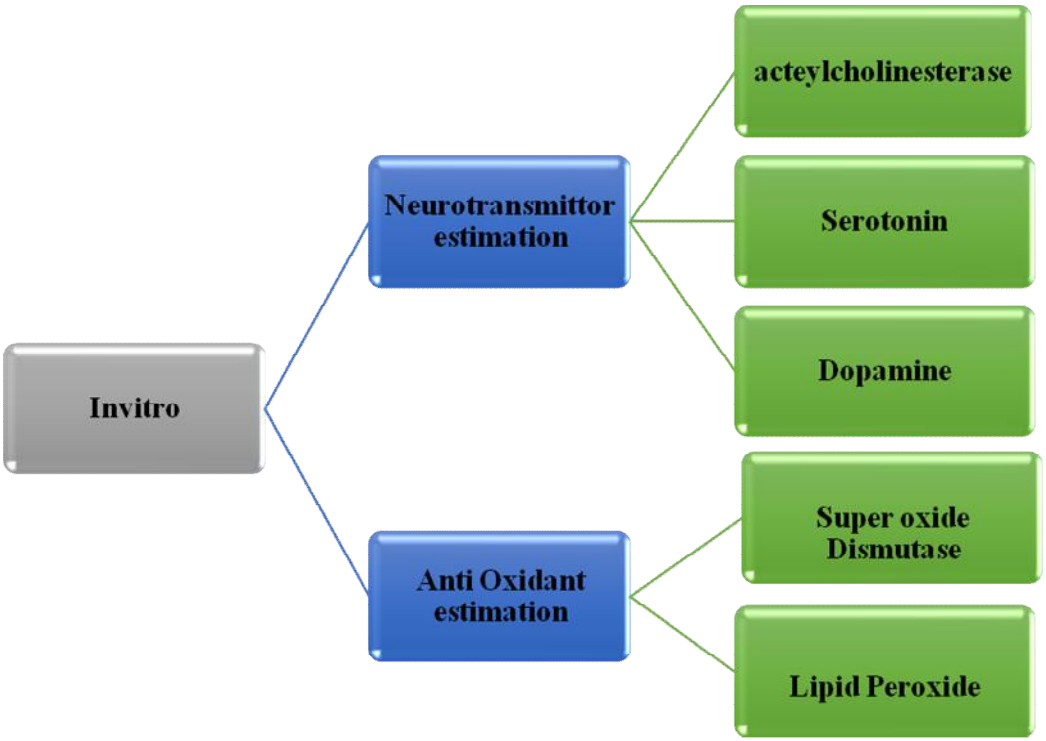
6. PLAN OF WORK



`


InVivo
Elevated Zero Maze
Hole Board
Actophotometer
Marble Burying Behavior
Light and Dark test


Histopathological Study
Brain
Kidney
Liver


Invitro
Neurotransmittor estimation
Anti Oxidant estimation
acteylcholinesterase
Serotonin
Dopamine
Super oxide Dismutase
Lipid Peroxide

7. MATERIALS AND METHODS

7.1 COLLECTION

The marketed product vacha churna were bought from online shopping website **Ayus vastha**

7.2 PREPARATION OF VACHA CHURNAM:

The required amount of drug was dispersed in distilled water and was administered orally to mice by using intra-gastric catheter.

The preparation was freshly prepared on daily basis till the termination of experiment.

Fig. No: 4 Vacha Churnam

8. PRELIMINARY PHYTOCHEMICAL ANALYSIS [111]

Vacha churna was subjected to preliminary phytochemical screening for the presence or absence of phytoconstituents by the following methods.

1. TEST FOR ALKALOIDS:

Vacha Churna was treated with diluted HCl and filtered. The filtrate was treated with various alkaloidal agents.

Mayer's Test: Sample was treated with Mayer's reagent; appearance of cream color indicates presence of alkaloids.

**Dragendroff`s Test**: Sample was treated with Dragendroff`s reagent, appearance of reddish brown precipitate indicates presence of alkaloids.

2. TEST FOR CARBOHYDRATES:

**Fehling`s Test:** To the sample Fehling`s solution A and B was added and heated for two minutes. Appearance of reddish brown color indicates presence of reducing sugars.

3. TEST FOR PROTEINS:

Biuret`s Test: To Vacha Churna, add copper sulphate ($CuSO_4$) solution followed by sodium hydroxide solution, a violet color precipitates indicates presence of proteins.

4. TEST FOR STEROIDS:

Liebermann Burchard`s Test:

Vacha Churna was treated with concentrated Sulphuric acid (H_2SO_4) and glacial acetic acid followed by acetic anhydride, a violet ring appears at the junction of the liquids and appearance of green color in the aqueous layer indicates presence of steroids.

5. TEST FOR PHENOLS:

Vacha Churna was treated with neutral ferric chloride solution and few drops of alcohol, appearance of bluish green or red color indicates presence of phenols.

6. TEST FOR TANNINS:

Vacha Churna was treated with 10% lead acetate solution appearance of white precipitate indicates presence of tannins.

7. TEST FOR FLAVONOIDS:

Vacha Churna was hydrolyzed with 10%sulphuric acid and cooled. It was then extracted with diethyl ether and divided in to 3 portions in three separate test tubes.1ml of diluted sodium carbonate, 1ml of 0.1 N sodium hydroxide and 1 ml of diluted ammonia solutions were added to the first, second and third test tube respectively. Development of yellow color in each test tube indicates presence of flavonoids.

Shinoda's test:

Vacha Churna dissolved in alcohol, to which a piece of magnesium followed by drop wise addition of concentrated hydrochloric acid and heated. Appearance of purple color indicates presence of flavonoids.

8. TEST FOR GLYCOSIDES:

Vacha Churna was mixed with a little anthrone on a while on a watch glass.one drop concentrated Sulphuric acid was added made into a paste and warmed gently over water bath.

9. TEST FOR SAPONINS:

Foam test: Vacha Churna was diluted to 20 ml with distilled water, formation of foam in the upper part of the test tubes presence of saponins.

10. TEST FOR TERPENES:

Vacha Churna were treated with tin and thionyl chloride, appearance of pink color indicates presence of Terpenes.

9. ACUTE TOXICITY STUDIES: [112]

The acute toxicity was done by using OECD guidelines 423.The acute toxic class method (423) was step wise procedure with 3 animals of single sex per step. Depending on the mortality and/or morbidity status of the animals, on the average 2-4 steps may be necessary to allow judgment on the acute toxicity of the substances. This procedure results in the use of a specified number of animals while allowing for acceptable data- based scientific conclusion.

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

9.1 PROCEDURE:

Adult female Swiss albino mice weighing 20- 30gms were used for the study. The starting dose level of 2000mg/kg body weight p.o of vacha churna was given. Since most of the crude extracts possess LD50 value more than 2000 mg/kg, p.o. so starting dose 2000mg/g p.o. was used. Dose volume administered was 1ml/100 gm body weight to mice which were fasted overnight with water *ad libitum*. Food was withheld for further 3-4hrs after oral administration of drugs and observed for the signs of toxicity.

Body weight of each mice before and after administration of vacha churna was noted and any changes in skin and fur, eyes and mucous membrane, respiratory, circulatory, autonomic & central nervous system, motor activity and behavior pattern was observed and also sign of tremors, convulsion, salivation, diarrhoea, lethargy, sleep and coma was noted. The onset of toxicity and signs of toxicity was also noted.

10. EXPERIMENTAL ANIMALS

The Swiss albino mice weighing 22-30gm were used for this study. The inbreed animals were procured from the animal house of C.L. Baid Metha college of pharmacy, Thoraipakkam, Chennai- 97.They were housed six per kg under standard laboratory conditions at a temperature 22±2°C with 12:12 hrs light and dark circle. The animals were provided with standard animal feed, water and *ad libitum*. The animals were adapted to laboratory conditions one week prior to initiation of experiments. All experiments were carried out according to the guidelines for care and use of experimental animals and approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The study was approved by Institutional Animal Ethical Committee (IAEC)

10.1 EXPERIMENTAL DESIGN:

On the first day of experiment the animals were divided randomly into five groups of six animals each. Anxiety is induced by low temperature-induced stress for the II, III, IV, V groups and continued for 10 days.

Control animals were given distilled water orally by using intragastric catheter, the last dose was given 60 min prior to behavioral testing and on 21st day sacrification of animal were done for in vitro studies.

IAEC REFERENCE NO: IAEC/LI/01/CLBMCP/2017

10.2 GROUPING

S.NO	GROUPS	TREATMENT
1	Group I	Control
2	Group II	Negative Control
3	Group III	Standard (Diazepam 5mg/kg,i.p)
4	Group IV	Low dose (200mg/kg,p.o)
5	Group V	High dose (400mg/kg,p.o)

11. METHODS AND ASSESSMENTS

11.1 LOCOMOTOR ACTIVITY TEST:

- Locomotor activity (horizontal activity) was measured using **Actimeter**. Actophotometer which operates on photoelectric cells connected with a counter.
- When a beam of light falling on the photocell is cut off by the animal a count is recorded and displayed digitally.
- Each mice was placed individually in the activity cage floor for 10 min and the locomotion count was directly read from the digital reading displayed in the actimeter.
- Actimeter is the combination of hole board and actophotometer in which both rats and mice can be placed.

11.2 ELEVATED ZERO MAZE: [113]

- Elevated zero maze is a modification of the elevated plus maze model of anxiety in mice, which incorporates both traditional and novel ethological measures in the analysis of drug effects.
- The elevated zero-maze constitutes a modification in both design and procedure which aims to improve upon the traditional model by increasing the sensitivity to, and facilitating interpretation of, drug action.
- The novel design comprises an elevated annular platform with two opposite, enclosed quadrants and two open quadrants, and addresses one potentially problematic issue inherent in the plus-maze design, i.e., the unavoidable ambiguity associated with time spent on the central square, removing any ambiguity in the interpretation and allowing uninterrupted exploration.
- Transfer latency was defined as the time (in seconds) taken by the animal to move from the open arm into one of the covered arms with all its four legs.

11.3 DARK-LIGHT COMPARTMENT: [114]

- The apparatus used for the light/dark transition test consisted of a cage (21x42x25 cm) divided into two sections of equal size by a partition with door.
- Mice are housed six per cage in a room with a 12 hr light/dark cycle (lights on at 7:00 A.M.) with ad libitum access to food and water.

- All the cages containing rats are transferred to the behavior testing room 30 min before the first trial begins.
- One chamber is brightly illuminated by white diodes (390 lux), whereas the other chamber is dark (2 lux).
- Mice are placed into the dark side and the door is opened manually 3 seconds.
- The door is used so that the mice do not enter the light chamber immediately after the release with their motivation to escape from experimenter, since the latency to enter the light chamber may serve as an index of anxiety-like behavior.
- Mice are allowed to move freely between the two chambers with door open for 10 min.
- The total number of transitions, the time spent in the each chamber, no of rearings and the latency to enter the light chamber is recorded manually.
- After each trial, all chambers are cleaned with super hypochlorous water to prevent a bias based on olfactory cues.

11.4 MARBLE BURYING BEHAVIOR: [115]

- Place one mice into a corner of the cage containing marbles, being careful to place the mice on bedding as far from marbles as possible, and place the filter-top cover on the cage.
- Withhold food and water during the test. Allow mice to remain in the cage undisturbed for 30 min.
- Remove the mice and return it to its home cage after test completion, taking extreme care not to move or dislodge the marbles in the process of removing the subject from the cage.
- Score a marble as buried if two-thirds of its surface area is covered by bedding.
- Average scores for the number of marbles buried for each mice was recorded manually. Retrieve all 20 marbles from the bedding. Dispose of bedding.

12. NEUROTRANSMITTERS ESTIMATION [116]

PREPARATION OF TISSUE EXTRACTS:

REAGENTS :

- HCl-butanol
- Heptanes
- 0.1M HCl: (0.85ml conc. HCl upto 100ml of water)

PROCEDURE:

On the day of experiment mice were sacrificed, whole brain was dissected out and the subcortical region (including the striatum) was separated. Tissue was weighed and homogenized in 5ml HCl-butanol for about 1 min followed by the sample was then centrifuged for about 10 min at 2000rpm. An aliquot supernatant phase (1ml) was removed and added to Eppendorf tube containing 2.5ml heptane and 0.31ml of 0.1M HCl. After 10 min of vigorous shaking, the tube was centrifuged under the same conditions as above in order to separate two phases, and the overlaying organic phase was discarded. The aqueous phase (0.2ml) was then taken for the dopamine, serotonin and norepinephrine assay. All the steps were carried out at 0°C. it was taken in between 50-75 mg of the tissue for homogenate with 5ml of HCl-butanol in correlation of same tissue concentration of 0.1ml of HCl-butanol in Schlumpf method.

12.1 ESTIMATION OF DOPAMINE:

REAGENTS:

- 0.4M HCl: 3.4ml conc. HCl upto 100ml water
- Sodium acetate buffer pH (6.9)
- 5M NaOH
- 0.1M Iodine solution (in ethanol)
- Sodiumthiosulphate
- 10M acetic acid: 57ml of glacial acetic acid dissolved in distilled water upto 100ml.

PROCEDURE:

To the 0.2ml of aqueous phase, 0.5ml 0.4M HCl and 0.1ml of EDTA/sodium acetate buffer (pH6.9) were added, followed by 0.1ml iodine solution (0.1M in ethanol) in oxidation. The reaction was stopped after 2 min by addition of 0.1ml Sodium thiosulphate solution. 0.1ml acetic acid is added after 1.5 min. the solution was then heated to 100°C for 6 min when the sample again reached room temperature, excitation and emission spectra were read from the spectrofluorimeter. The readings were taken at 330-370nm.

$$\textit{Unknown Conc.} = \frac{\textit{Intensity of unknown} * \textit{Conc of Std}}{\textit{Intensity of Std}}$$

12.2 ESTIMATION OF SEROTONIN:

The serotonin content was estimated by the method of Schlumpf.

REAGENTS:

- O-Phthalaldialdehyde (OPT) reagent

PROCEDURE:

To 0.2ml of aqueous extract 0.25ml of OPT reagent was added. The flurophore was developed by heating to 100°C for 10 min. after the samples reached equilibrium with the ambient temperature, readings were taken at 360-470nm in the spectrofluroimeter. For serotonin tissue blank, 0.25ml conc. HCl without OPT was added. Internal standard: 500µg /ml each of noradrenaline, dopamine and serotonin are prepared in distilled water: HCl-butanol in 1:2 ratios.

$$\textit{Unknown Conc.} = \frac{\textit{Intensity of unknown} * \textit{Conc of Std}}{\textit{Intensity of Std}}$$

12.3 DETERMINATION OF ACETYLCHOLINESTERASE (ACHE) ENZYME [117]

Acetylcholinesterase enzyme activity was estimated by Elman method.

REAGENTS

1. 0.1M phosphate buffer
2. DTNB Reagent
3. Acetylthiocholine (ATC)

PROCEDURE

The mice were decapitated,brains are removed quickly and placed in ice cold saline. Frontal cortex, hippocampus and septum are quickly dissected out on a petri dish chilled on crushed ice.the tissues were weighed and homogenized in 0.1M phosphate buffer(pH 8).0.4ml aliquot of the homogenate is added to a cuvette containing 2.6ml of phosphate buffer(0.1M,pH 8) and 100µl of DTNB. The contents of the cuvette are mixed thoroughly by bubbling air and absorbance is measured at 412nm in a spectrophotometer. When absorbance reaches a stable value,it is recorded as basal reading.20µl of substrate i.e., acetylthiocholine is added and change in absorbance is recorded. Change in absorbance/min is thus determined.

Reagents	**Sample**	**Blank**
Phosphate buffer solution	2.6ml	2.7ml
Supernatant	0.4ml	0.4ml
DTNB	0.1ml	……

CALCULATIONS

The enzyme activity is also calculated by using the following formula

$$\text{Acetylcholinesterase activity } (M/ml) = \frac{A/min \times Vt}{\varepsilon \times b \times VS}$$

where,

A/min= Change in absorbance per min

ε= 1.361x 104 M/cm

b= path length (1cm)

Vt= total volume (3.1ml)

VS= sample volume (0.4ml)

The final reading of enzyme activity is expressed as µ moles/min/mg tissue

$$\textbf{\textit{µ moles/min/mg protein}} = \frac{\textbf{\textit{µ moles/ml sample}}}{\textbf{\textit{mg protein/ml sample dilution}}}$$

13. ESTIMATION OF ANTIOXIDANT ENZYME

13.1 ESTIMATION OF SUPEROXIDE DISMUTASE (SOD) [118]

REAGENTS:

- Carbonate buffer (100mM, pH 10.2)
- Epinephrine (3mM)

PROCEDURE:

The SOD activity in supernatant was measured by the method of Misra and Fridovich. The supernatant (500µl) was added to 0.800ml of carbonate buffer (100mM, pH 10.2) and 100µl of epinephrine (3mM). The change in absorbance of each sample was then recorded at 480nm in spectrophotometer for 2min at an interval of 15sec. Parallel blank and standard were run for determination of SOD activity.

One unit of SOD is defined as the amount of enzyme required to produce 50% inhibition of epinephrine auto oxidation.

Reagents	Uninhibited (Standard)	Inhibited (Sample)	Blank
Carbonate buffer	**0.900ml**	**0.800ml**	**1.0ml**
Supernatant	**-**	**0.1ml**	**-**
Epinephrine	**0.1ml**	**0.1ml**	**-**

The reaction mixtures are diluted 1/10 just before taking the readings in spectrophotometer

13.2 ESTIMATION OF LIPID PEROXIDASE (LPO) [119]

The level of Lipid peroxidase was estimated by Thiobarbituric acid reaction method described by Ohkawa *et al.*

REAGENTS

1. Sodium dodecyl sulphate (SDS) (8.1% w/v)
2. Acetic acid (20%; pH 3.5)
3. Thiobarbituric acid (TBA) (0.8%)
4. n-butanol/pyridine mixture (15:1)

PROCEDURE

To 0.2ml of test sample, 0.2ml of SDS, 1.5ml of acetic acid and 1.5ml of TBA were added. The mixture was made up to 4ml with water and then heated in a water bath at 95°C for 60min. After cooling, 1ml of water and 5ml of n-butanol/pyridine mixture were added and shaken vigorously. After centrifugation at 4000rpm for 10min, the organic layer was taken and its absorbance was read at 532nm. The level of lipid peroxides was expressed as nmoles of MDA released/g wet tissue.

Reagents	Sample	Blank
SDS	0.2ml	0.2ml
Supernatant	0.2ml	-
DDW	1.6ml	1.8ml
Acetic acid	1.5ml	1.5ml
TBA	1.5ml	1.5ml
n-butanol/pyridine	5ml	5ml

14. METHODS FOR HISTOPATHOLOGICAL STUDY

The mice from each group were anaesthetized using intra peritoneal injection of thiopentone sodium. The brain was carefully removed without any injury after opening the skull. liver and kidney are also dissected carefully without any damage to the tissues. The collected organs were washed with ice cold normal saline and fixed in 10% buffered neutral formalin. The tissues were processed for routine paraffin embedding and 5 micron sections were stained with Mayer's Haematoxylin Eosin stain.

15. STATISTICAL ANALYSIS

The statistical analysis was carried by one way ANOVA followed by Dunnet's ―t‖ test. P values <0.05 (95% confidence limit) was considered statistically significant, using Software Graph pad Prism 6.0

16. TABLES AND GRAPHS

TABLE 1: PHYTOCHEMICAL SCREENING OF VACHA CHURNAM

S.NO	CONSTITUENTS	REMARKS
1	Alkaloids	Present
2	Carbohydrates	Present
3	Protein	Present
4	Steroids	Present
5	Phenols	Present
6	Tannins	Present
7	Flavonoids	Present
8	Glycosides	Present
9	Saponins	Present
10	Terpenes	Present

TABLE 2: EFFECT OF VACHA CHURNAM IN ACUTE TOXICITY STUDY

S. No	Treatment	Dose	Weight of animal (g)		Signs of Toxicity	Onset of Toxicity	Reversible or Irreversible	Duration
			Before test	After test				
1.	Vacha Churnam	2000mg/kg	25	28	No signs of toxicity	Nil	Nil	14 days
2.	Vacha Churnam	2000mg/kg	20	22				
3.	Vacha Churnam	2000mg/kg	20	22				

TABLE 3: EFFECT OF VACHA CHURNAM IN ELEVATED ZERO MAZE:

S.NO	GROUP	% TIME SPENT IN OPEN ARM	% ENTRIES IN OPEN ARM
1	Control	2.50±0.86	11.25±1.90
2	Negative Control	1.05±0.68	9.75±4.30
3	Standard	11.17±1.58	37.7±4.72
4	Vacha churna 200mg/kg	7.12±0.63	30.8±0.68
5	Vacha churna 400mg/kg	10.61±3.65	35.35±4.46

Values are represented in Mean ± SEM, n=6

Comparison: a- Group III vs Group IV and Group V

Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test.

ns- Non significant, *p<0.05, **p<0.01, ***p<0.001

GRAPH 1: EFFECT OF VACHA CHURNAM IN ELEVATED ZERO MAZE:

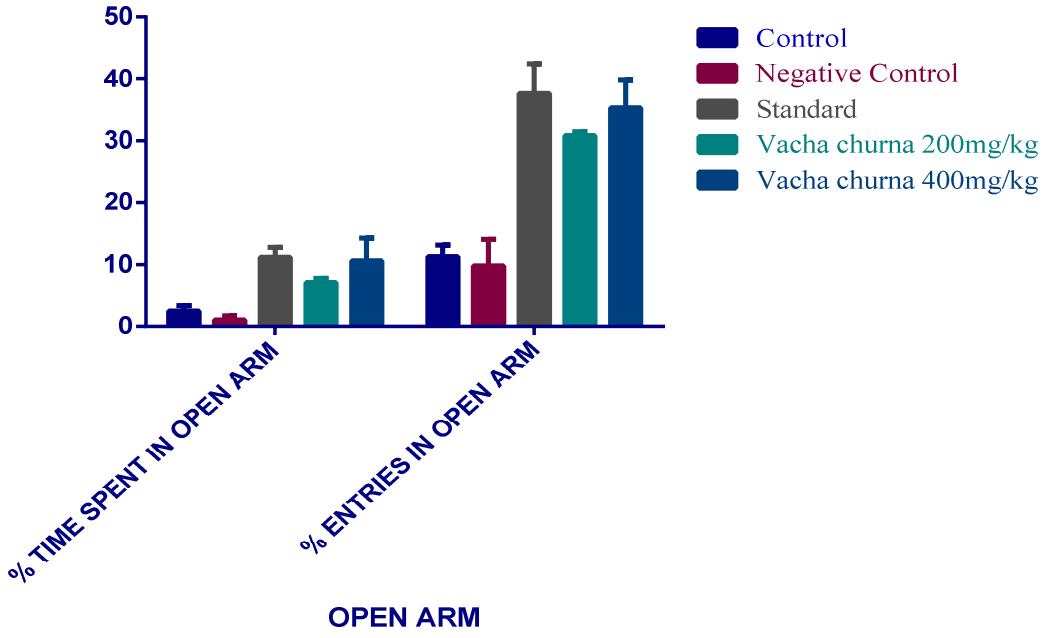


TABLE 4: EFFECT OF VACHA CHURNAM IN LIGHT-DARK TEST:

S.NO	GROUP	TIME SPENT IN LIT AREA (Sec)	NO. OF TRANSITIONS
1	Control	49.67±4.90	19.83±1.25
2	Negative Control	30.26±3.31	17±1.28
3	Standard	101.83±5.08	31.33±1.91
4	Vacha churna 200mg/kg	47±4.80	23.83±2.41
5	Vacha churna 400mg/kg	70.00±5.97	27.93±3.30

Values are represented in Mean ± SEM, n=6

Comparison: a- Group III vs Group IV and Group V

Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test.

ns- Non significant, *p<0.05, **p<0.01, ***p<0.001

GRAPH 2: EFFECT OF VACHA CHURNAM IN LIGHT-DARK TEST:

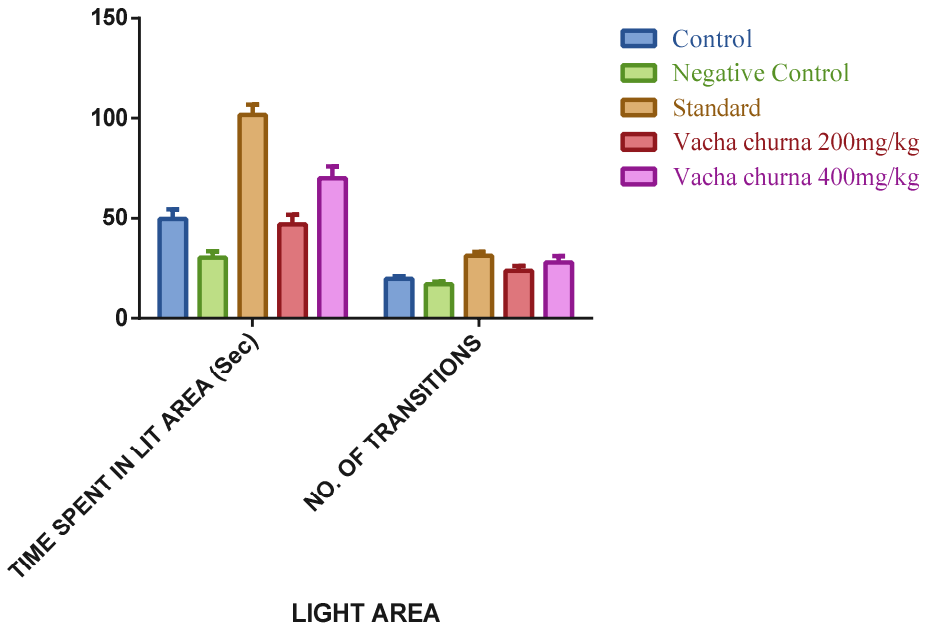


TABLE 5: EFFECT OF VACHA CHURNAM IN MARBLE BURYING BEHAVIOR:

S.NO	GROUP	NO. OF MARBLES BURIED
1	Control	16.75±0.57
2	Negative Control	18.78±0.67
3	Standard	6.25±0.63
4	Vacha churna 200mg/kg	14.25±0.47
5	Vacha churna 400mg/kg	10.25±0.25

Values are represented in Mean ± SEM, n=6

Comparison: a- Group III vs Group IV and Group V

Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test.

ns- Non significant, *p<0.05, **p<0.01, ***p<0.001

GRAPH 3: EFFECT OF VACHA CHURNAM IN MARBLE BURYING BEHAVIOR:

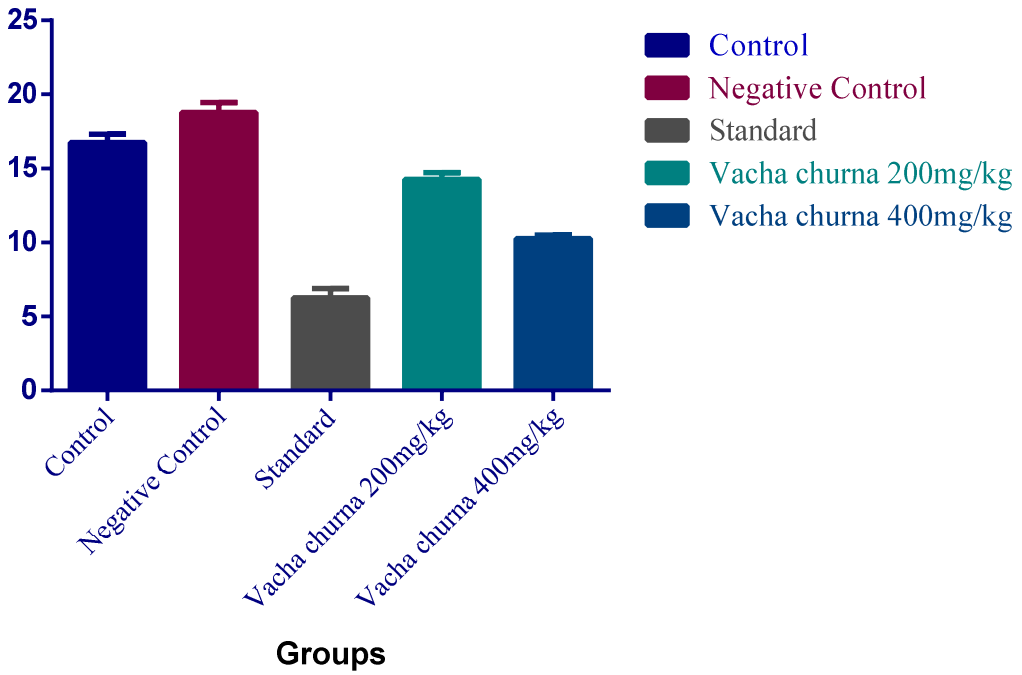


TABLE 6: EFFECT OF VACHA CHURNAM IN MORRIS WATER MAZE:

S.NO	GROUP	ESCAPE LATENCY(SEC)
1	Control	15.8±2.3
2	Negative Control	59.3±7.3
3	Standard	11.02±5.3
4	Vacha churna 200mg/kg	16.0±2.6
5	Vacha churna 400mg/kg	13.0±1.6

Values are represented in Mean ± SEM, n=6

Comparison: a- Group III vs Group IV and Group V

Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test.

ns- Non significant, *p<0.05, **p<0.01, ***p<0.001

GRAPH 4: EFFECT OF VACHA CHURNAM IN MORRIS WATER MAZE:

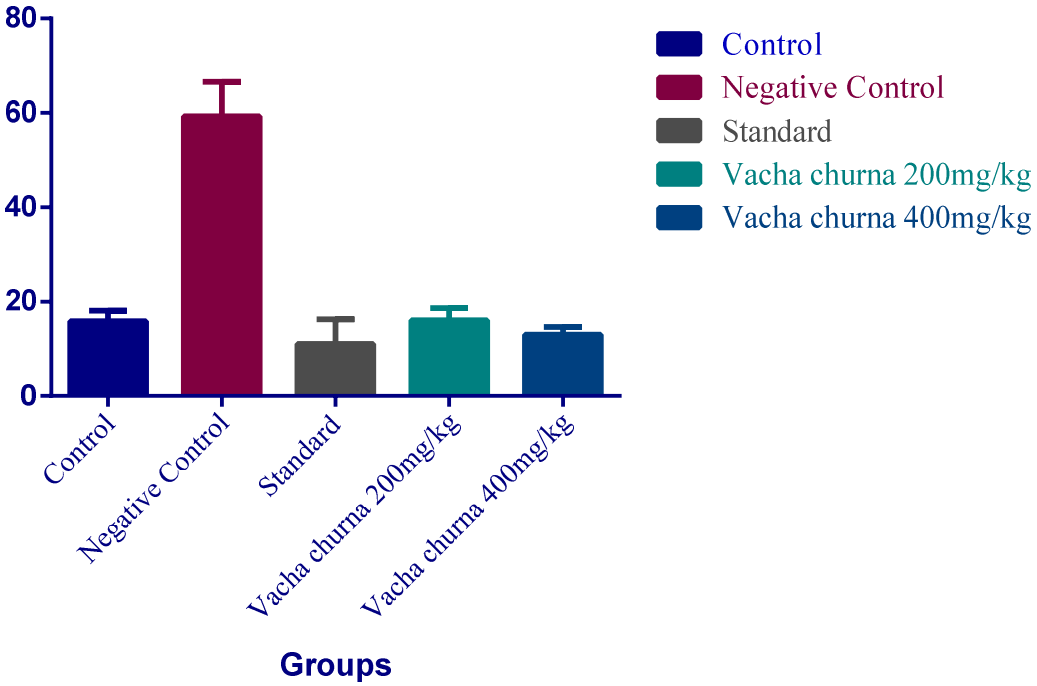


TABLE 7: EFFECT OF VACHA CHURNAM IN ACTOPHOTOMETER:

S.NO	GROUP	LOCOMOTOR ACTIVITY
1	Control	357.75±5.39
2	Negative Control	459.75±3.49
3	Standard	110±5.35
4	Vacha churna 200mg/kg	259.5±7.59
5	Vacha churna 400mg/kg	156.75±3.83

Values are represented in Mean ± SEM, n=6

Comparison: a- Group III vs Group IV and Group V

Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test.

ns- Non significant, *p<0.05, **p<0.01, ***p<0.001

GRAPH 5: EFFECT OF VACHA CHURNAM IN ACTOPHOTOMETER:

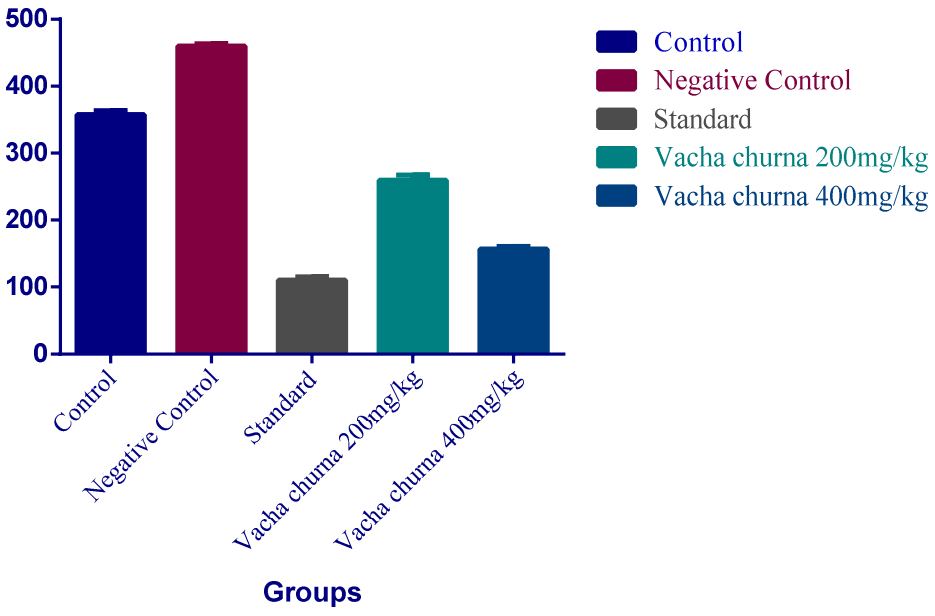


TABLE 8: EFFECT OF VACHA CHURNAM IN HOLE BOARD:

S.NO	GROUP	NO. OF LINE CROSSING	NO. OF HEAD DIPPING	NO. OF REARING
1	Control	66.25±2.46	22.5±1.5	10.25±0.75
2	Negative Control	82±1.78	25.5±1.8	12.25±0.95
3	Standard	26.5±1.55	8.7±0.5	1.75±0.75
4	Vacha churna 200mg/kg	56.5±1.19	17.75±0.94	8±0.41
5	Vacha churna 400mg/kg	41.5±3.17	12±0.40	5.25±0.48

Values are represented in Mean ± SEM, n=6

Comparison: a- Group III vs. Group IV and Group V

Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test.

ns- Non significant, *p<0.05, **p<0.01, ***p<0.001

GRAPH 6: EFFECT OF VACHA CHURNAM IN HOLE BOARD:

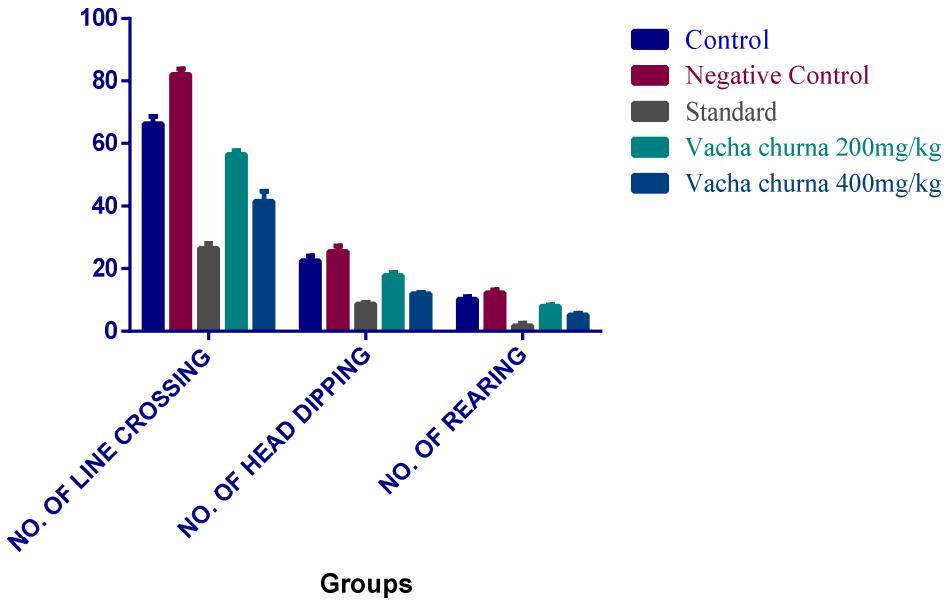


TABLE 9: EFFECT OF VACHA CHURNAM IN ACETYLCHOLINESTERASE (ACHE)

S.NO	GROUP	ng/mg WET TISSUE
1	Control	25.63±0.57
2	Negative Control	12.34±0.67
3	Standard	23.34±0.12
4	Vacha churna 200mg/kg	15.34±0.2
5	Vacha churna 400mg/kg	20.98±0.11

Values are represented in Mean ± SEM, n=6

Comparison: a- Group III vs Group IV and Group V

Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test.

ns- Non significant, *p<0.05, **p<0.01, ***p<0.001

GRAPH 7: EFFECT OF VACHA CHURNAM IN ACETYLCHOLINESTERASE (ACHE):

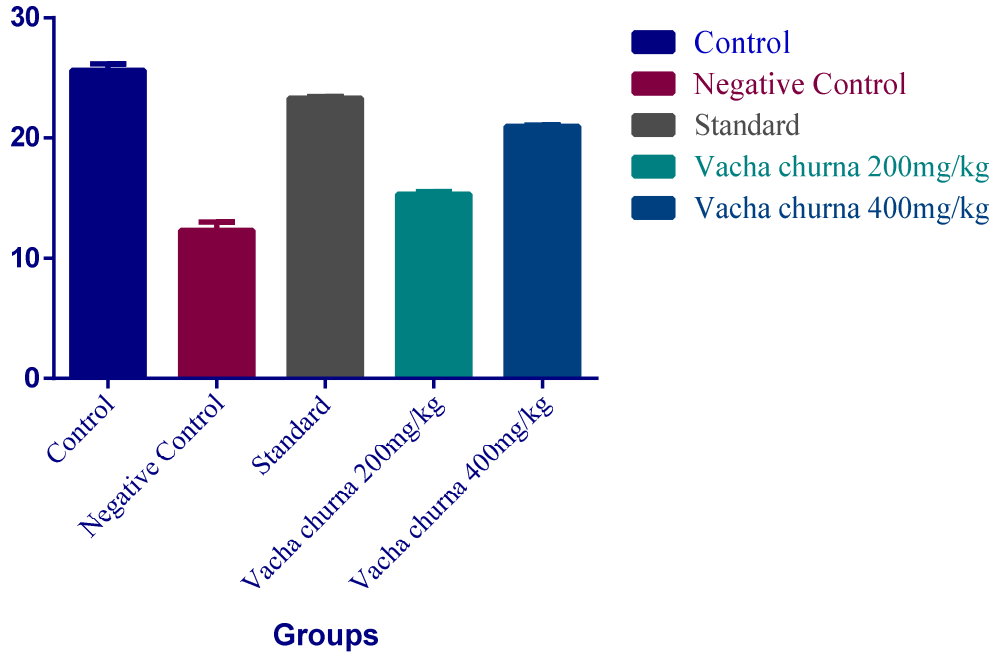


TABLE 10: EFFECT OF VACHA CHURNAM IN DOPAMINE

S.NO	GROUP	ng/mg WET TISSUE
1	Control	430.23±20.34
2	Negative Control	308.45±2.45
3	Standard	400.23±3.2
4	Vacha churna 200mg/kg	239.47±2.55
5	Vacha churna 400mg/kg	381.45±4.34

Values are represented in Mean ± SEM, n=6

Comparison: a- Group III vs Group IV and Group V

Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test.

ns- Non significant, *p<0.05, **p<0.01, ***p<0.001

GRAPH 8: EFFECT OF VACHA CHURNAM IN DOPAMINE

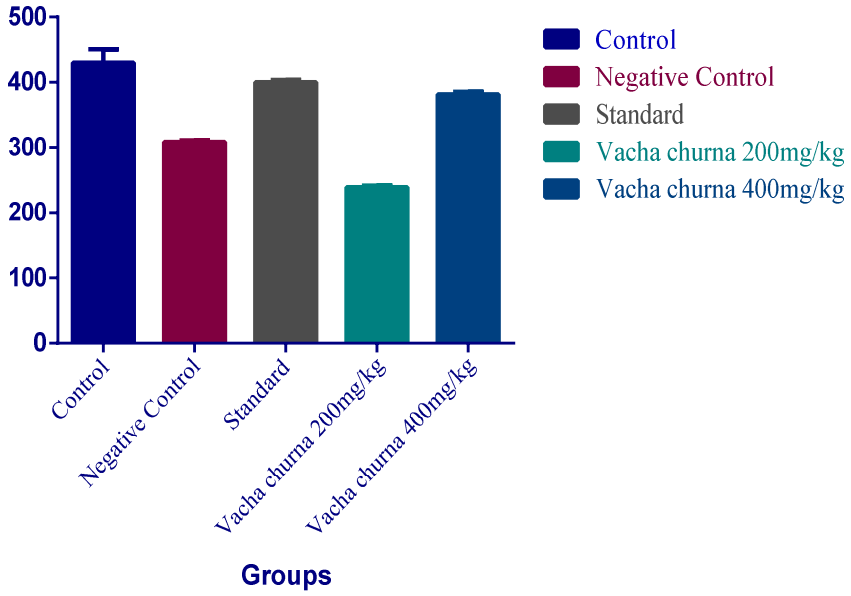


TABLE 11: EFFECT OF VACHA CHURNAM IN SEROTONIN:

S.NO	GROUP	ng/mg WET TISSUE
1	Control	243.34±5.6
2	Negative Control	181.33±5.34
3	Standard	235.55±5.32
4	Vacha churna 200mg/kg	203±2.72
5	Vacha churna 400mg/kg	225.34±2.89

Values are represented in Mean ± SEM, n=6

Comparison: a- Group III vs Group IV and Group V

Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test.

ns- Non significant, *p<0.05, **p<0.01, ***p<0.001

GRAPH 9: EFFECT OF VACHA CHURNAM IN SEROTONIN:

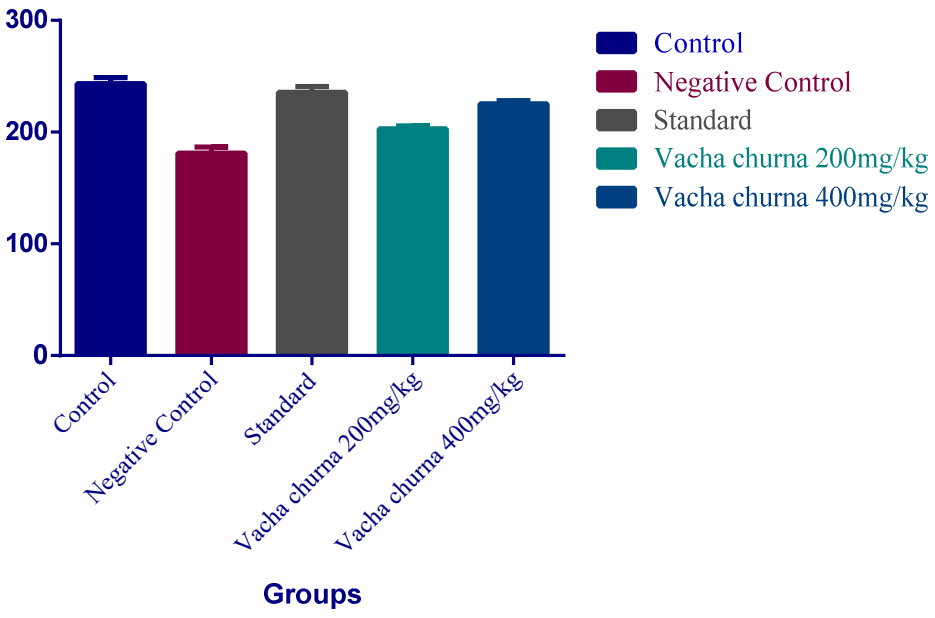


TABLE 12: EFFECT OF VACHA CHURNAM IN SUPEROXIDE DISMUTASE (SOD):

S.NO	GROUP	Units/mg wet tissue
1	Control	9.32±0.4
2	Negative Control	4.1±0.25
3	Standard	9.02±0.41
4	Vacha churna 200mg/kg	5.99±0.3
5	Vacha churna 400mg/kg	7.187±0.21

Values are represented in Mean ± SEM, n=6

Comparison: a- Group III vs Group IV and Group V

Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test.

ns- Non significant, *p<0.05, **p<0.01, ***p<0.001

GRAPH 10: EFFECT OF VACHA CHURNAM IN SUPEROXIDE DISMUTASE (SOD):

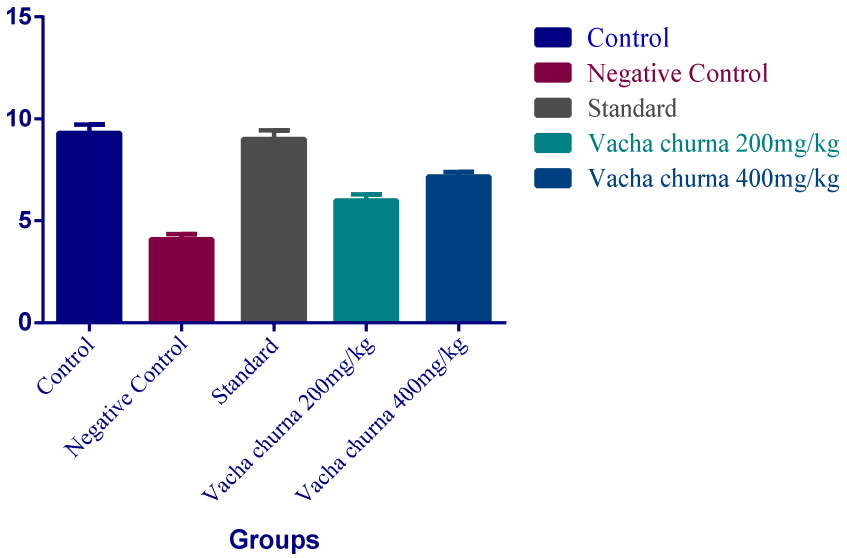


TABLE 13: EFFECT OF VACHA CHURNAM IN LIPID PEROXIDASE (LPO):

S.NO	GROUP	Units/mg wet tissue
1	Control	70.1±0.3
2	Negative Control	109.3±0.23
3	Standard	72.34±1.3
4	Vacha churna 200mg/kg	95.45±1.23
5	Vacha churna 400mg/kg	80.23±1.32

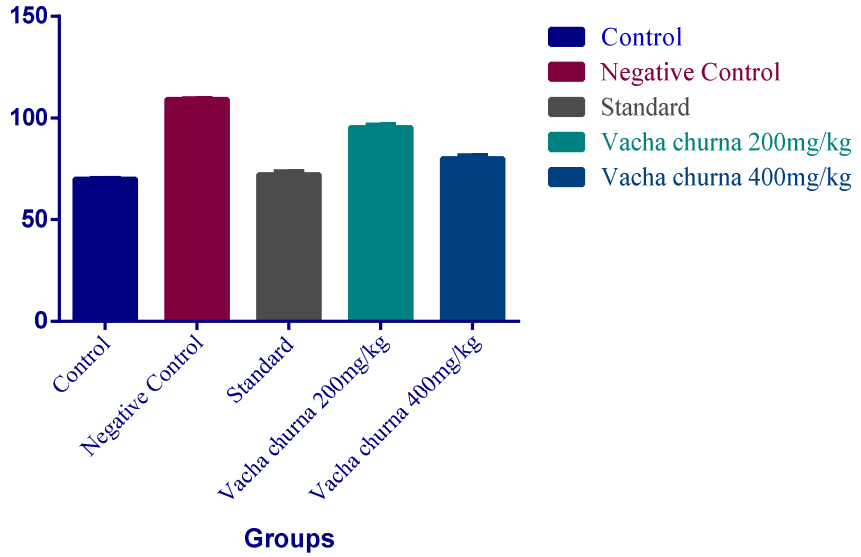
Values are represented in Mean ± SEM, n=6

Comparison: a- Group III vs Group IV and Group V

Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test.

ns- Non significant, *p<0.05, **p<0.01, ***p<0.001

GRAPH 11: EFFECT OF VACHA CHURNAM IN LIPID PEROXIDASE (LPO):



17. RESULTS

17.1 PRELIMINARY PHYTOCHEMICAL ANALYSIS

The preliminary phytochemical analysis on Vacha Churnam revealed the presence of various phytoconstituents including alkaloids, carbohydrates, protein, steroids, phenols, tannins, flavonoids, etc. which are given in Table 1.

17.2 ACUTE ORAL TOXICITY STUDIES

The acute oral toxicity was done according to OECD 423(acute toxic class method) guidelines. A single administration of starting dose of 2000mg/kg of body weight p.o. of Vacha Churna was administered to the three female mice and observed for three days. There was no change in the body weight before and after treatment of the experiment and no sign of toxicity were observed. Observations are shown in Table 2

17.3 IN VIVO PARAMETERS

17.3.1 EFFECT OF VACHA CHURNAM IN ELEVATED ZERO MAZE

The Group II animals showed decreased in percentage of alteration when compared with Group I animals. Treatment with Vacha Churna (200 and 400 mg/kg) showed ($p<0.05$ and $p<0.01$ for Groups IV and V respectively) increase time spent duration in open arm when compared with group II which was comparable with that of standard. Results are given in Table 3 and plotted in Graph 1.

17.3.2 EFFECT OF VACHA CHURNAM IN LIGHT-DARK TEST

The Group II animals showed decrease in time spent in light compartment when compared with Group I animals. Treatment with Vacha Churna (200 and 400 mg/kg) showed ($p<0.05$ and $p<0.01$ for Groups IV and V respectively) increase time spent duration in light compartment when compared with group II which was comparable with that of standard. Results are given in Table 4 and plotted in Graph 2.

17.3.3 EFFECT OF VACHA CHURNAM IN MARBLE BURYING BEHAVIOR

The Group II animals buried more no. of marbles when compared with Group I animals. Treatment with Vacha Churna (200 and 400 mg/kg) showed ($p<0.05$ and $p<0.01$ for Groups IV and V respectively) decreased burying behavior when compared with group II which was comparable with that of standard. Results are given in Table 5 and plotted in Graph 3.

17.3.4 EFFECT OF VACHA CHURNAM IN MORRIS WATER MAZE

The escape latency of group II was much higher when compared with group I. Treatment with Vacha Churna (200 and 400 mg/kg) showed ($p<0.05$ and $p<0.01$ for Groups IV and V respectively) decreased escape latency on hidden platform when compared with group II which was comparable with that of standard. Results are given in Table 6 and plotted in Graph 4.

17.3.5 EFFECT OF VACHA CHURNAM IN ACTOPHOTOMETER

The Group II animals showed significant increase in locomoter activity when compared with Group I animals. Treatment with Vacha Churna (200 and 400 mg/kg) showed decrease in locomoter activity statistically ($p<0.05$ and $p<0.01$ for Groups IV and V respectively) and Group III also showed decreased activity when compared with Group II. Results are given in Table 7 and plotted in Graph 5.

17.3.6 EFFECT OF VACHA CHURNAM IN HOLE BOARD

The no. of line crossing, head dipping and rearing of group II was decreased when compared with group I. Treatment with Vacha Churna (200 and 400 mg/kg) showed ($p<0.05$ and $p<0.01$ for Groups IV and V respectively) increased no. of line crossing, head dipping and rearing when compared with group II which was comparable with that of standard. Results are given in Table 8 and plotted in Graph 6.

17.4 NEUROTRANSMITTERS ESTIMATION

17.4.1 EFFECT OF VACHA CHURNAM IN ACETYLCHOLINESTERASE

The brain AchE level in group II was significantly decreased when compared with group I. Treatment with Vacha Churna (200 and 400 mg/kg) showed ($p<0.05$ and $p<0.01$ for Groups IV and V respectively) increased AchE levels in brain when compared with group II which was comparable with that of standard. Results are given in Table 9 and plotted in Graph 7.

17.4.2 EFFECT OF VACHA CHURNAM IN DOPAMINE

The brain dopamine level in group II was significantly decreased when compared with group I. Treatment with Vacha Churna (200 and 400 mg/kg) showed ($p<0.05$ and $p<0.01$ for Groups IV and V respectively) increased dopamine levels in brain when compared with group II which was comparable with that of standard. Results are given in Table 10 and plotted in Graph 8.

17.4.3 EFFECT OF VACHA CHURNAM IN SEROTONIN

The brain serotonin level in group II was significantly decreased when compared with group I. Treatment with Vacha Churna (200 and 400 mg/kg) showed ($p<0.05$ and $p<0.01$ for Groups IV and V respectively) increased serotonin levels in brain when compared with group II which was comparable with that of standard. Results are given in Table 11 and plotted in Graph 9.

17.5 ESTIMATION OF ANTIOXIDANT ENZYME

17.5.1 EFFECT OF VACHA CHURNAM IN SUPEROXIDE DISMUTASE

The SOD levels in brain in group II was significantly decreased when compared with group I. Treatment with Vacha Churna (200 and 400 mg/kg) showed ($p<0.05$ and $p<0.01$ for Groups IV and V respectively) increased SOD levels in brain when compared with group II which was comparable with that of standard. Results are given in Table 12 and plotted in Graph 10

17.5.2 EFFECT OF VACHA CHURNAM IN LIPID PEROXIDASE

The LPO levels in group II was significantly increased when compared with group I. Treatment with Vacha Churna (200 and 400 mg/kg) showed ($p<0.05$ and $p<0.01$ for Groups IV and V respectively) decreased LPO levels when compared with group II which was comparable with that of standard. Results are given in Table 13 and plotted in Graph 11.

17.6 ASSESSMENT OF HISTOPATHOLOGICAL CHANGES

It was observed that there was decrease in density of neuronal cells and disrupted in the normal distribution of neuronal cells in Group II animals with respect to Group I animals. Treatment groups (Group III, IV, V) exhibited improved neuronal configuration than Group II. Group IV and V showed significant improvement in the density of neuronal cells of brain when compared with neuronal loss in negative control group (Group II). Whereas Group III showed improvement in the density of neuronal cells. Histopathological pictures are shown in

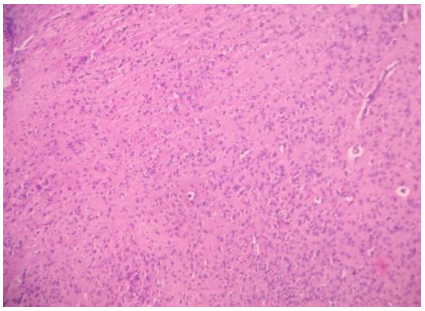


Fig. 5: Control

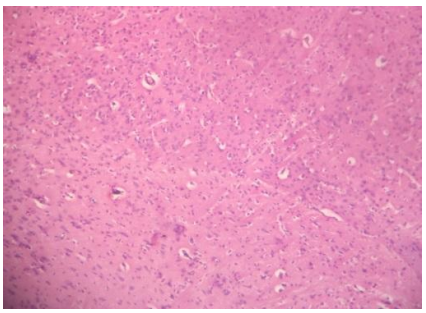


Fig. 6: Standard

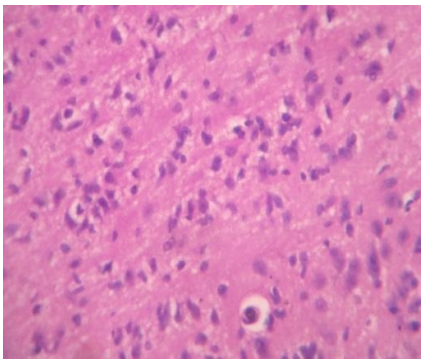


Fig. 7: Negative Control

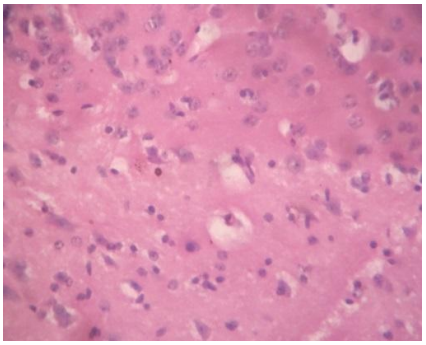


Fig. 8: Low Dose

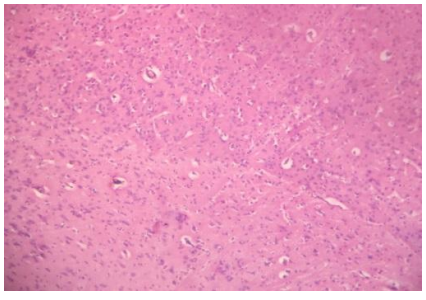


Fig. 9: High Dose

18. DISCUSSION

Benzodiazepines 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, credible benefits and moderate costs are of interest, especially in primary care settings.

Medicinal plants and herbal therapy like Ayurveda, Siddha, Unani are a good source to find new remedies for these disorders. In the search for an alternative, more specific, and perhaps cost-free therapy, research has been conducted to investigate natural anxiolytic drugs.

The effects of 200 and 400 mg/kg of Vacha churnam on the Elevated zero maze, light–dark test, actophtometer, morris water maze, hole board etc. were almost equivalent to that of 4mg/kg diazepam.

In the present study, the anxiolytic activity of Vacha Churnam was observed at doses of 200 and 400 mg/kg in mice. These observations clearly indicate that Vacha churnam exerts an anxiolytic activity.

Anxiolytic activity of Vacha churnam is likely to be associated with essential constituents present in it reported in plant description and literature review. It is reported that vacha churnam exhibited anti-convulsant properties, and anxiolytic and sedative effects.

Literature review has suggested that many clinical trials have been conducted and it was reported that vacha churnam exhibited many properties in CNS disorders.

Vacha churnam is also used for the management of CNS disorders. Vacha churnam may be a useful in the management of neurodegenerative diseases, such as Alzheimer's disease, on account of its multifarious effects, that is, memory-improving property and anticholinesterase activity

In summary, Vacha churna has an anxiolytic activity.

19. CONCLUSION

The selected dose 200mg/kg and 400mg/kg of Vacha Churnam showed significant action in memory and retention, cognitive, anxiolytic behavior but higher dose 400mg/kg showed better action than lower dose 200mg/kg.

Literature review concluded that vacha churnam exhibited many activities in CNS disorders. So, I conclude that vacha churnam is effective in anxiety and anxiety related behavior.

It showed potent depletion in anxiety related parameters and had very good response in in vitro studies such as serotonin, AchE, dopamine, SOD etc.

Further pharmacological and chemical investigations are required to elucidate the exact mechanism of action of this extract and to isolate the active principles responsible for such effects.

20. REFERENCES

1. York: W.W. Norton & Company.
2. Davison, Gerald C. (2008). Abnormal Psychology. Toronto: Veronica Visentin. p. 154. ISBN 978-0-470-84072-6.
3. Jump up to: a b c d American Psychiatric Association (2013). Diagnostic and Statistical Manual of Mental Disorders (Fifth ed.). Arlington, VA: American Psychiatric Publishing. p. 189. ISBN 978-0-89042-555-8.
4. Jump up Bouras, N.; Holt, G. (2007). Psychiatric and Behavioral Disorders in Intellectual and Developmental Disabilities (2nd ed.). Cambridge University Press.
5. 2005 - 2017 WebMD, LLC. http://www.webmd.com/anxiety-panic/guide/anxiety-disorders#1
6. Diagnostic and Statistical Manual of Mental DisordersAmerican Psychiatric Associati. (5th ed.). Arlington: American Psychiatric Publishing. 2013. pp. 189–195. ISBN 978-0890425558.
7. 2017 Health Media Ventures, Inc. *http://www.health.com/health/gallery/0,,20646990,00.html*
8. Lydiard RB: "The role of GABA in anxiety disorders". J Clin Psychiatry 2003; 64 (3): 21-27
9. Nemeroff CB: "The role of GABA in the pathophysiology and treatment of anxiety disorders". Psychopharmacology Bulletin 2003; 37(4):133-146.
10. Nutt DJ, Ballenger JC, Sheehan D and Wittchen HU: "Generalized anxiety disorder: comorbidity, comparative biology and treatment". J. Neuropsychopharmacol 2002; 5:315–325.
11. Sandford JJ, Argyropoulous SV and Nutt DJ: "The psychobiology of anxiolytic drugs". Part 1: Basic neurobiology. PharmacolTher 2000; 88:197-212.
12. Cloos JM and Ferreira V: "Current use of benzodiazepines in anxiety disorders". Current Opinion in Psychiatry, 2009; 22(1): 90–5.
13. Rudolph U, Crestani F and Mohler H: GABA (A) receptor subtypes: dissecting their pharmacological functions. Trends PharmacolSci 2001; 22(4): 188-94.
14. Juergens, MD, Steven M:"Understanding Benzodiazepines". California Society of Addiction Medicine. Retrieved 25 April 2012.

15. Bandelow B, Reitt M, Rover C, Michaelis S, Gorlich Y and Wedekind D: "Efficacy of treatments for anxiety disorders: a meta-analysis". International Clinical Psychopharmacology 2015; 30 (4): 183–92

16. O'Brien: "Benzodiazepine Use, Abuse, and Dependence". J Clin Psychiatry 2005; 66.

17. Dooley DJ, Donovan CM, Meder WP and Whetzel SZ: Preferential action of gabapentin and pregabalin at P/Q-type voltage-sensitive calcium channels: inhibition of K+/- evoked [3H]-norepinephrine release from rat neocortical slices. Synapse 2002;45:171-190

18. De-paris F, Busnello JV and Vianna MR: "The anticonvulsant compound gabapentin possesses anxiolytic but not amnesic effects in rats". BehavPharmacol 2000;11: 168-173.

19. Tirault M, Foucan L, Debaene B; "Gabapentin premedication: assessment of preoperative anxiolysis and postoperative patient satisfaction". ActaAnaesthesiolBelg 2010; 61: 203-9.

20. Field MJ, Oles RJ and Singh L: "Pregabalin may represent a novel class of anxiolytic agents with a broad spectrum activity". Br J Pharmacol 2001;132:1-4.

21. Ocanez KL, McHugh RK, Otto MW: "A meta-analytic review of the association between anxiety sensitivity and pain". Depress Anxiety 2010; 27: 760-7.

22. Hamner MB, Brodrick PS and Labbate LA: "Gabapentin in PTSD: a retrospective, clinical series of adjunctive therapy". Ann Clin Psychiatry 2001; 13:141-146.

23. Mula M, Pini S and Cassano GB: "The role of anticonvulsant drugs in anxiety disorders: a critical review of the evidence". J ClinPsychopharmacol 2007; 27 (3): 263–72.

24. Selak I: "Pregabalin" (Pfizer). CurrOpinInvestig Drugs 2001; 2:828-834.

25. Fink K, Dooley DJ and Meder WP: "Inhibition of neuronal Ca (2+) influx by gabapentin and pregabalin in the human neocortex". Neuropharmacol 2002;42:229-236.

26. Field MJ, Oles RJ and Singh L: "Pregabalin may represent a novel class of anxiolytic agents with a broad spectrum activity". Br J Pharmacol 2001;132:1-4.

27. Kasper S, Blagden M and Seghers S: "A placebo-controlled study of pregabalin and venlafaxine treatment of GAD". Paper presented at: Collegium InterantionaleNeuropsychopharmacologium Annual Meeting. 2001; Montreal, Canada; June 23-27.

28. Curtis DR and Watkins JC: "The excitation and depression of spinal neurones by structurally related amino acids". J Neurochem 1960;6: 117-41.

29. Lujan R, Shigemoto R and Lopez-Bendito G: "Glutamate and GABA receptor signalling in the developing brain". Neuroscience 2005; 130:567-580.

30. Lujan R, Shigemoto R and Lopez-Bendito G: "Glutamate and GABA receptor signalling in the developing brain". Neuroscience 2005; 130:567-580.

31. Aarts MM and Tymianski M: "Novel Treatment of Excitotoxicity: Targeted Disruption of Intracellular SignallingFrom Glutamate Receptors".Biochemical Pharmacology, 2003; 66 (6): 877–886.

32. Hynd MR, Scott HL and Dodd PR: "Glutamate-mediated excitotoxicity and neurodegeneration in Alzheimer's disease". NeurochemInt 2004; 45:583-595.

33. Meldrum BS: "Glutamate as a neurotransmitter in the brain: review of physiology and pathology". J Nutr 2000; 130(4):1007S-1015S.

34. Swanson C, Bures M, Johnson M, Linden A and Monn J: "Metabotropic Glutamate receptors as novel targets for anxiety and stress disorders," Nature Reviews Drug Discovery 2005; 131-144

35. Kew JN and Kemp JA: "Ionotropic and metabotropic glutamate receptor structure and pharmacology". Psychopharmacology (Berl) 2005; 179:4-29.

36. Konradi C and Heckers S: "Molecular aspects of glutamate dysregulation: implications for schizophrenia and its treatment". PharmacolTher 2003; 97:153–79.

37. RiazaBermudo-Soriano C, Puente García R, Chinchilla Moreno A, Rodríguez Quirós J and Vega Piñero M: "Neurodesarrollo, esquizotaxia, y modelodiátesis-estrésenesquizofrenia. Las esquizofrenias: sushechos y valoresclínicos y terapéuticos". Elsevier 2007; 75–99.

38. Bergink V, van Megen HJ and Westenberg HG: "Glutamate and anxiety". EurNeuropsychopharmacol 2004; 14:175–83.

39. WierońskA J: "The Loss of Glutamate-GABA Harmony in Anxiety Disorders" intechopen.com

40. Geha RS, Beiser A, Ren C, Patterson R, Greenberger PA, Grammer LC, Ditto AM, Harris KE, Shaughnessy NA, Yarnold PR, Corren J and Saxon A: "Review of alleged reaction to MSG and outcome of a multicentre double blind placebo-controlled study". J. Nutr 2000; 130:1058s-1062s.

41. He KL, Zhao Daviglus, ML, Dyer AR, Horn LV, Garside D, Zhu L, Guo D, Wu Y, Zhou B and Stamler J: "Association of monosodium glutamate intake with overweight in Chinese adults: the INTERMAP Study". Obesity 2008; 16(8): 1875–1880

42. Cortese BM and Phan LK: "The Role of Glutamate in Anxiety and Related Disorders". CNS Spectr 2005; 10(10): 820-830.

43. Husarova V and Ostatnikov D: "Monosodium Glutamate Toxic Effects and Their Implications for Human Intake: A Review". JMED Research 2013; Article ID: 608765.

44. Umukoro S, Oluwole GO, Olamijowon HE, Omogbiya AI and Eduviere AT: "Effect of Monosodium Glutamate on Behavioral Phenotypes, Biomarkers of Oxidative Stress in Brain Tissues and Liver Enzymes in Mice". World Journal of Neuroscience, 2015; 5, 339-349.

45. Velloso NA, GD Dalmolin, G Fonini, VD GindriSinhorin, A Ferreira da Silveira and MA Rubin: "Spermine attenuates behavioral and biochemical alterations induced by quinolinic acid in the striatum of rats". Brain Res 2008; 1198 :107–114.

46. Moschou PN, Roubelakis-Angelakis KA: "Polyamines and programmed cell death". Journal of Experimental Botany, 2013; 65: 1285–1296

47. Lawrence SA: Amines: synthesis, properties and applications. Cambridge University Press 2004; 64.

48. Pellegrini-Giampietro DE: "An activity-dependent spermidine-mediated mechanism that modulates glutamate transmission". Trends Neuroscience 2003; 26:9–11.

49. Minois N:"Molecular Basis of the "Anti-Aging" Effect of Spermidine and Other Natural Polyamines – A Mini-Review". Gerontology. 2014; 60 (4): 319–326.

50. Fiori LM,Turecki G: "Implication of the polyamine system in mental disorders". J Psychiatry Neurosci. 2008; 33(2): 102-10.

51. Rubin MA, Stiegemeier JA, Volkweis MA, Oliveira DM, Fenili AC, Boemo RL, Jurach A, Mello CF: "Intra-amygdala spermidine administration improves inhibitory avoidance performance in rats". Eur J Pharmacol,2001; 423: 35-39.

52. Pellegrini-Giampietro DE: "An activity-dependent spermidine-mediated mechanism that modulates glutamate transmission". Trends Neurosci26: 9-11.

53. AdibhatlaRM,Hatcher JF, Sailor K, Dempsey RJ: "Polyamines and central nervous system injury: spermine and spermidine decrease following transient focal cerebral ischemia in spontaneously hypertensive rats". Brain Res. 2002; 938(1-2): 81-6.

54. Rosenberg DR, MacMaster FP, Keshavan MS, Fitzgerald KD, Stewart CM and Moore GJ: Decrease in caudate glutamatergic concentrations in pediatric obsessive-compulsive disorder patients taking paroxetine. J Am Acad Child Adolesc Psychiatry 2000; 39:1096 – 1103.

55. Brunton LL, Chabner B, KnollmannBC.Goodman and Gilman's The Pharmacological Basis of Therapeutics (12th ed.). New York: McGraw-Hill Professional. 2011.

56. ZavodnickAD,Ali R: "Lamotrigine in the treatment of unipolar depression with and without comorbidities: a literature review". Psychiatr Q. 2012; 83(3): 371-83.

57. Berlant J and van Kammen DP: Open-label topiramate as primary or adjunctive therapy in chronic civilian posttraumatic stress disorder: a preliminary report. J Clin Psychiatry 2002; 63:15–20.

58. Jia M and Pittman J: "Deficits in striatal Dopamine and Hippocampal serotonin following induction of anxiety/Depressive like behaviours by Bisphenol A", Arch neuroscience 2014; 2(2):1-6.

59. Graeff FG: "On serotonin and Experimental anxiety"; Psychopharmacology 2002; 163:467-476.

60. Lesch KP and Gutknecht L: "Pharmacogenetics of the serotonin transporter". ProgNeuropsychopharmacolBiol Psychiatry 2005; 29(6):1062-73.

61. Golimbet VE, Alfimova MV and Mitiushina NG: "Polymorphism of the serotonin 2A receptor gene (5HTR2A) and personality traits". MolBiol 2004; 38(3): 404-12

62. Gordon JA and Hen R: "Genetic approaches to the study of anxiety". Annu Rev Neurosci 2004; 27:193-222

63. Neumeister A, Young T and Stastny J: "Implications of genetic research on the role of the serotonin in depression: emphasis on the serotonin type 1A receptor and the serotonin transporter". Psychopharmacology (Berl) 2004; 174(4):512-24.

64. Bert B, Schmidt N, Voigt JP, Fink H and Rex A: "Evaluation of cage leaving behaviour as a free choice paradigm". Journal of Pharmacological and Toxicological Methods under revision, 2011.

65. Adell A: "Lu-AA21004, a multimodal serotonergic agent, for the potential treatment of depression and anxiety". IDrugs 2010; 13(12): 900-10.

66. Stahl SM, Grady MM, Moret C and Briley M: "SNRIs: their pharmacology, clinical efficacy, and tolerability in comparison with other classes of antidepressants". CNS Spectr 2005; 10(9): 732-47.

67. Thase ME: "Treatment of anxiety disorders with venlafaxine XR". Expert Rev Neurother.2006; 6(3):269-82.

68. Burgdorf J, Panksepp J, Beinfield MC, Kroes RA and Moskal JR: "Regional brain cholecystokinin changes as a function of rough and tumble play behavior in adolescent rats"; Peptides 2006; 27:172-177.

69. Chen Q, Nakajima A, Meacham C, Tang YP: "Elevated cholecystokininergic tone constitutes an important molecular/neuronal mechanism for the expression of anxiety in the mouse". Proc Natl AcadSci 2006; 103:3881-

70. HajizadehMA,Hosseini RS, Roohbakhsh A: "Anxiogenic effect of CCK8s in the ventral hippocampus of rats: possible involvement of GABA(A) receptors". Pharmacol Rep. 2012; 64(1): 45-53.

71. Del BC,Lutz PE, Le MJ, Koebel P, Kieffer BL: "Cholecystokinin knock-down in the basolateral amygdala has anxiolytic and antidepressant-like effects in mice". 2012; 218:185-95.

72. Latini S and Pedata F: "Adenosine in the central nervous system: release mechanisms and extracellular concentrations". J Neurosci 2001; 79:463-

73. Sachdeva S and Gupta M: "Adenosine and its receptors as therapeutic targets: An overview", Saudi Pharmaceutical Journal 2013; 21:245-253.

74. MaximinoC,Lima MG, Olivera KR, Picanço-Diniz DL, Herculano AM: "Adenosine A1, but not A2, receptor blockade increases anxiety and arousal in Zebrafish". Basic ClinPharmacolToxicol. 2011; 109(3):203-7.

75. Coelho JE, Alves P, Canas PM, Valadas JS, Shmidt T, Batalha VL, Ferreira DG, Ribeiro JA, Bader M, Cunha RA, do Couto FS and Lopes LV: "Overexpression of adenosine A2A receptors in rats: effects on depression, locomotion, and anxiety". Psychiatry,2014; 5:67.

76. Bert B, Schmidt N, Voigt JP, Fink H and Rex A: "Evaluation of cage leaving behaviour as a free choice paradigm"; Journal of Pharmacological and Toxicological Methods under revision 2011.

77. Prediger R, Silva G, Batista L and Takahashi R: "Activation of Adenosine A_1 Receptors reduces anxiety like behavior during acute ethanol withdrawal (hangover) in mice", Neuropsychopharmacology 2006; 31:2210-2220.

78. Deepak M, Tripathi AS, Wadhwani PJ, Shriao AV and Chandewar AV: "Neurobiological modulators of anxiety", Interantional Research Journal of Research 2012; 3(1):60-64.

79. Dall S: "Plant-derived Acetylcholinesterase inhibitory alkaloids for the treatment of Alzheimer's disease," 2013:19-28.

80. Mineur S, Obayemi A and Wigestrand B: "Cholinergic signaling in the hippocampus regulates social stress resilience and anxiety and depression like behavior", 2013; 110(9):3573-3578.

81. Anderson SM, Brunzell DH; "Low Dose Nicotine and Antagonism of β2 Subunit Containing Nicotinic Acetylcholine Receptors Have Similar Effects on Affective Behavior in Mice". PLoS ONE, 2012; 7(11).

82. Collins F: "Anxiety Reduction: Exploring the Role of Cannabinoid Receptors". NIH Director's Blog, April 2014.

83. Ashwani A, Tarun K, Malik A and Hooda A: "Anxiety disorder: A review", International Research Journal of Pharmacy 2011; 2(5):18-23.

84. Ned HK, Andrew SF, Rothem K, Marissa KR, Eva MF, Patrick HR, Do PMT, Benjamin PG, Miles EO, Ethan KB, Daniel RM, Andrew LA, Marina EE, Walter FB, Julie LF, Jonathan AO: "Overexpressing Corticotropin-Releasing Factor in the Primate Amygdala Increases Anxious Temperament and Alters Its Neural Circuit". Biological Psychiatry, 2016; 80 (5).

85. Comai S and Gobbi G: "Unveiling the role of Melatonin MT2 receptors in sleep, Anxiety and other neuropsychiatric diseases: a novel target in Psychopharamacolgy". Journal of Psychiatry Neuroscience 2014; 39(1):6-21.

86. Reardon LE, Leen F and Hayward C: "A critical review of the empirical literature on the relation between anxiety and puberty". Clinical Psychology Review, 2009; 29, 1-23.

87. *https://www.news-medical.net/health/Diagnosis-of-Anxiety.aspx*

88. Mental Health Facts and Statistics. NIMH Website. National Institute of Mental Health, n.d. Web. 20 Feb. 2013.

89. "antianxiety agent" at Dorland's Medical Dictionary

90. Youngstedt, Shawn D.; Kripke, Daniel F. (2007). "Does bright light have an anxiolytic effect – an open trial". BMC Psychiatry. 7: 62. PMC 2194679 Freely accessible. PMID 17971237. doi:10.1186/1471-244X-7-62.

91. Hayes, Peggy E.; Schulz, S. Charles (1987). "Beta-blockers in anxiety disorders". Journal of Affective Disorders. 13 (2): 119–30. PMID 2890677. doi: 10.1016/0165-0327(87)90017-6.

92. "anxiolytic (tranquilizer)". Memidex (WordNet) Dictionary/Thesaurus. Retrieved 2010-12-02.

93. Galanter, Marc (1 July 2008). The American Psychiatric Publishing Textbook of Substance Abuse Treatment (American Psychiatric Press Textbook of Substance Abuse Treatment) (4 ed.). American Psychiatric Publishing, Inc. p. 197. ISBN 978-1-58562-276-4.

94. Mechanism Of Action Of Anxiolytics By John F. Tallman James Cassella John Kehne

95. *http://www.calmclinic.com/anxiety/drugs/side-effects*

96. *http://mentalhealthdaily.com/2015/02/26/5-new-anxiety-medications-in-development-2015/*

97. *http://www.pherin.com/products.html*

98. *http://www.ncbi.nlm.nih.gov/pubmed/22178753*

99. *Reference: https://bebrainfit.com/natural-remedies-anxiety/*

100. *https://www.news-medical.net/health/Treatment-of-Anxiety.aspx*

101. *http://dx.doi.org/10.1590/1516-4446-2013-1139*

102. Gordon JA and Hen R: "Genetic approaches to the study of anxiety". Annu Rev Neurosci 2004; 27:193-222.

103. Rasayana: Ayurvedic Herbs for Longevity and Rejuvenation H.S. Puri CRC Press, 29-Aug-2003 - Health & Fitness - 368 pages

104. Selye H (1983). The stress concept: past, present, and future, In, Stress Research, Issues for the Eighties, ed. By C L Cooper, (pp.1–21) John Wiley & Sons, New York.

105. Selye H (1946). General adaptation syndrome and diseases of adaptation. J ClinEndocrinol. 6, pp. 117–230

106. Dahanukar, S A (1986). Study of influence of plant products on Adaptive Processes, PhD thesis, Dept of Pharmacology and therapeutics, University of Mumbai, Mumbai.

107. Ader R, Felten D and Cohen M (1990). Interactions between the brain and the immune system, Ann. Rev. Pharmacol. Toxicol. (pp. 30, 561–602.)

108. Shastri, K. Rastrangni by Sharma, Sadanand, (translation of Sanskrit text into Hindi). MotilalBanarsi Das, Delhi, India 1979.

109. Hoffmann, D., The Herbal Handbook. Healing Arts Press, Rochester, Vermont 1998.

110. *https://www.ayurtimes.com/vacha-sweet-flag-acorus-calamus/*

111. C.K.Kokate, A.P.Purohit, S.B.Gokhale.Text book of Pharmacognosy47thedition. August 1, 2007.

112. *https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd_gl423.pdf*

113. Kamila et al., IJPSR, 2015; Vol. 6(1): 300-307. E-ISSN: 0975-8232; P-ISSN: 2320-5148

114. *https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2504462/*

115. Marble Burying and Nestlet Shredding as Tests of Repetitive, Compulsive-like Behaviors in Mice Mariana Angoa-Pérez1, Michael J. Kane1, Denise I. Briggs1, Dina M. Francescutti1, Donald M. Kuhn1

116. Schlumpf M, Lichtensteiger W, Langemann H, Waser PG, Hefti F. A fluorometric micromethod for the simultaneous determination of serotonin, noradrenaline and dopamine in milligram amounts of brain tissue. Biochemical pharmacology. 1974 Sep 1;23(17):2437-46.

117. Aebi H. Methods of enzymatic analysis, ed. Newyork*, Academic press, 1972, 2:674*.

118. Kono Y. Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. Archives of biochemistry and biophysics. 1978 Feb 1;186(1):189-95.

119. Ohkawa H, Ohisi N and Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction*. AnnalBiochen, 1979; 95: 351-358*.