TO STUDY THE ATTENTION DEFICIT HYPERACTIVITY DISORDER (ADHD) AND TO EVALUATE THE COGNITIVE, HYPERACTIVE AND IMPULSIVE BEHAVIOR AGAINST 6-OHDA HBr LESIONED SPRAGUE DAWLEY NEONATES USING *HYPERAXE, CURCUMIN & QUERCETIN*

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

R ASHISH JAIN
REGISTRATION No: 261525001

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 **To study the Attention Deficit Hyperactivity Disorder (ADHD) and to evaluate the cognitive, hyperactive and impulsive behavior against 6-OHDA HBr lesioned Sprague Dawley neonates using *HYPERAXE, CURCUMIN & QUERCETIN*** submitted by Regn No: **261525001** in partial fulfilment 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 **To study the Attention Deficit Hyperactivity Disorder (ADHD) and to evaluate the cognitive, hyperactive and impulsive behavior against 6-OHDA HBr lesioned Sprague Dawley neonates using *HYPERAXE, CURCUMIN & QUERCETIN*** submitted by Regn No: **261525001** in partial fulfilment 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: **261525001** hereby declare that this dissertation entitled, **To study the Attention Deficit Hyperactivity Disorder (ADHD) and to evaluate the cognitive, hyperactive and impulsive behavior against 6-OHDA HBr lesioned Sprague Dawley neonates using *HYPERAXE, CURCUMIN & QUERCETIN*** 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 at any other university.

Place : Chennai

Date :

Register No: **261525001**

ACKNOWLEDGEMENTS

Firstly I am very thankful to my respective guide **Dr.P.MURALIDHARAN, M.Pharm., Ph.D., Professor and Head Of Department** in Department of Pharmacology, C. L. BaidMetha College of Pharmacy, Chennai – 97. I am honoured to work under his guidance and have his precious guidelines, constant encouragement and support throughout my research work. I am very obliged for the patience with which he guided me at every step of my research work.

I consider it as a great honour to express my deep sense of gratitude and indebtedness to our Principal, **Dr. Grace Rathnam.**, M Pharm., Ph. D, of C.L Baid Metha College of Pharmacy, Chennai-97 for providing the necessary facilities to carry out this work.

I submissively express my deep sense of gratitude and sincere thanks to **Mr. Clement Atlee M.Pharm.,** Assistant Professor and Animal house in-charge, Department of Pharmacology, C.L.Baid Metha College of Pharmacy, Chennai-97, for his encouragement and timely provision of animals to carry out and complete this work.

I wish to express my sincere thanks to **Dr. P.Amudha M.pharm.PhD**, Assistant Professor of C.L. Baid Metha College of Pharmacy, Department of Pharmacology, Chennai -97, for her guidance regarding my dissertation work.

I wish to express my sincere thanks to **Dr. T.Purushoth Prabhu M.pharm.PhD**, **Professor and Head Of Department** in Department of Pharmacognosy, C. L. BaidMetha College of Pharmacy, Chennai – 97.for his guidance regarding my dissertation work.

I extend my special thanks to **Mr.Srinivasa Ragavan, M.Com,** store in-charge and attender **Mr. Anand**, **Mr. Rajaram,** C.L.Baid Metha College of Pharmacy, Chennai, for their timely help and supply of all necessary chemicals required for my project work and i also extend my thanks to our security incharge **Mr.GaneshBahadur.**

I extend my sincere thanks to Chief Librarian **M. Rajalakshmi,** C.L.Baid Metha

College of Pharmacy in helping me to utilize the library facilities for references.

I cannot ignore the support and help which I continuously received from my dear Mom and Dad **Kusum & Rajender** and my dear friends M. Pharmacy classmates **Monica shetty, Ramya kuttivelu, keerthi, Neha, Kajol, Akshit, Muthukumaran, Harika** and my brother **Anand** whose help, moral support helped me in completion of my project work.

Last but not least on behalf of humanity I want to thank personally to all those animals which were involved in this study.

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

R. ASHISH JAIN

LIST OF CONTENTS:

LIST OF FIGURES:

LIST OF TABLES:

LIST OF GRAPHS:

ABBREVATIONS:

5CSRT	Choice Serial Reaction Time Task
5-HIAA	5 Hydroxy Indole Acetic Acid
6-OHDA HBr	6-Hydroxydopamine hydrogen bromide
a.m	ante meridiem
AD	After Death
ADD	Attention Deficit Disorder
ADHD	Attention Deficit Hyperactive Disorder
AIDS	Aquired Immuno Deficiency Syndrome
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
APA	American Psychiatric Assosiation
ASD	Autistic Spectrum Disorder
ATP	Adenosine Tri-Phosphate
BDNF	Brain-derived neurotrophic factor
C	Celcious
Ca	Calcium
CAMP	Cyclic Adenosine Mono-Phosphate
CAR	Conditioned avoidance response
CD	Controlled Dellivery

cm	Centimeter
CNS	Central Nervous System
COMT	Catechol-O-methyltransferase
CPCSEA	Committee for the Purpose of Control And Supervision of Experiments on Animals
CS	Conditioned Stimulus
DA	Dopamine
DACC	Dorsal Anterior Cingulate Cortex
DAT	Direct Antiglobulin Test
DBH	Dopamine beta-hydroxylase
DOI	Digital Object identifier
DRD	Dopa-Responsive Dystonia
DSM	Diagnostic and Statistical Manual of Mental Disorder
EDTA	Ethylene Diamine Tetra Acetic Acid
EEG	Electroencephalogram
ER	Extended release
FDA	Food and Drug Administration
FMRI	Functional magnetic resonance imaging
GHS	Globally Harmonized System
Gi	Inhibitory G Protien

Gs	Stimulatory Gprotien
Hcl	Hydrochloric acid
HCN	Hyperpolarization-activated Cyclic Nucleotide-gated
HKD	Hyper kinetic disorder
HTR	head-twitch response
IAEC	International AnimalmEthical Committee
ICD	Interntional classification of diseases
ICV	Intra Cerebro Ventricular
IP	Intra Peritonial
IQ	Intelligent Quotient
KO	Knock out
LC	Locus coeruleus
LPHN	Latrophilin
LTP	Long term potentiation
luc	Illuminance
M	Molarity
MA	milli Ampere
MAO_A	Monoamine oxidase
MBD	Minimal Brain Disorder

MEG	Magneto encephalography
ml	milliliter
Mm	milli Molar
MOA	Mechanism of action
mRNA	messenger Ribo Nucleic Acid
N,S,E,W	North, South, East, West
NA	Nor-Adrenaline
NADPH	Nicotinamide adenine dinucleotide phosphate
NC	North Carolina
NDRI	Norepinephrine Dopamine reuptake Inhibitor
NE	Norepinephrine
NET	Norepinephrine reuptake Inhibitor
NHE	Naples High Exitability rat
NIRSI	Near Infrared Spectroscopic Imaging
NMDA	N-methyl-D-aspartate receptor
NO	Nitric Oxide
NRI	Norepinephrine reuptake inhibitor
NW	North West
ODT	Orally Disintegrating Tablets

OECD	Oraganisation for Economic Co-operation and Developement
OFC	Orbbito Frontal Cortex
PCB	Polychlorinated biphenyls
PET	Positron Emission Tomography
PFC	prefrontal cortex
Ph	Power of Hydrogen/ Potential of Hydrogen
p.o	Per os
PND	Post Natal Day
RDC	Research Domain Criteria
ROS	Reactive Oxygen Species
S	South
SD	Sprague Dawley
SEM	Standarad Error mean
SERT	Serotonin transporter
SHR	Spontaneously hypertensive rats
SNAP	synaptosomal nerve-associated proteins
SNRIs	Serotonin–norepinephrine reuptake inhibitor
SOD	Super Oxide Dismutase
SQUIDS	Superconducting Quantum Interference Device

SR	Sustained Release
SSRIs	Selective serotonin reuptake inhibitor
Std	Standard
SW	South west
TAAR	Trace amine-associated receptor
TPH	Tryptophan hydroxylase
US	Unconditioned stimulus
US	United States
USA	United States of America
USDD	United States Department of Defense
VMAT	Vesicular monoamine transporter
VTA	Ventral Tegmental Area
W	Watts
WHO	World Health Oranganization
WKHA	Wistar Kyoto hyperactive rats
WKY	Wistar-Kyoto normotensive
XR	Extended Release
α	Alpha
β	Beta

1. INTRODUCTION

Nature is the delightful example for the phenomena of symbiosis. Natural products originated from plants, animals, metals and minerals serving as the basis for the treatment of human disease. Medicinal plants based on tradition system of medicine have been playing an incredible role in providing diagnosis and treatment of human beings especially in developing countries. Utilization of herbal drug has also increased in developed countries. [1]

Herbal drug is the oldest form of health care known to mankind. Herbs have been used by all the cultures throughout the history. In modern civilization herbal drug is an integral part of the development. Primitive man observed and appreciated the great diversity of plants available to him. The most use of medicinal plant has been developed through observation of wild animal by trials and errors. As time moved on, each tribe added the medicinal power of herbs in their area based on their knowledge. They collected the information on herbs based on the method and well-defined it in herbal pharmacopoeia. Indeed, well into the 20th century most of the pharmacopoeia of scientific medicine was derived from the herbal lore of native place. Much of the drugs commonly use now a day is of herbal origin. Most civilized country USA dispensed about 25% of prescription which contains at least one active ingredient derived from plant materials. Some are made from plant extract others are synthesized to mimic the natural plant compounds. [2]

From last five thousand years human being have relied on natural product as the primary source of medicines. However, the last two centuries have brought an explosion to understand how the natural products are produced and how they react with other organisms. The World Health Organization (WHO) estimates that 80% of the world health populations presently use herbal medicines for some aspect of primary health care. [3]

In recent years synthetic drugs are showing more adverse effect, to overcome this problem researchers are trying to avoid this risk of those drugs. Whenever a drug is prescribed to a patient they are facing risk of side effect, so long term use of these drugs, patient should be careful. But in herbal medicine the toxic effects are negligible, so the uses of herbal drugs are growing up. Indians, Chinese are using plants as medicine, as whole plant or its extract. Toxicity of herbal drugs is less when compared with the synthetic medicines.[4]

India is known as an herbal garden in world and the largest producer of herbal medicines. India recognizes more than 3000 plants as medicinal use. It is estimated that more than 6000 plants in

India are in use in traditional and herbal system of medicines. Herbal medicines are used in various forms in indigenous system such as Unani, Ayurveda, and Siddha.[5]

Around 25,000 effective herbal formulations are used in traditional and folk medicine in India. The demand for plant products is increased throughout the world and the pharmaceutical companies are currently carrying out research on plant material for the potential medicinal components. Even though they are not able to prove the therapeutic effects of many plants, research continues to screen the active ingredients which form the basis of drugs to fight disease like psychological disorder, neurodevelopmental disorder, diabetes, cancer, AIDS and various more chronic diseases. [6]

In past years, the use of herbal drugs against various diseases is commonly non-toxic and have less side effects has developed. Even the World Health Organization (WHO) has recommended the effective use of plants in conditions wherever modern drugs are not safe.[7] Sometimes, herbal preparation produces a good therapeutic response when given in combination with allopathic drugs.

The CNS disorders cause a range of complex, distress and life threatening symptoms some of which are not at all responsive. They generally leave the patient unable to function normally Neuro transmitters cause neuro disorders such as ADHD, ASD, Parkinsonism disease, myasthenia gravis, epilepsy, anxiety myotropic lateral disease, multiple sclerosis.[8]

It is very important to use these medicines in a rational way. On basis of traditional knowledge about medicinal use of plant as therapeutic agents a rational approach has been developed to use medicinal plants as lead for discovery of active molecules e.g. Gingko biloba extract can be as CNS stimulant.[9]

In the modern research literature a number of clinical and experimental studies examine the potential herbs and formulae for ADHD and related conditions. The herbal literature contains herbal treatments for disorders that have symptoms and signs similar to those of ADHD by combining and systematically evaluating the data derived from the modern and classical literature, it is expected that the herbs and formulae with the greatest potential for further research can be identified.

Considering the advantages of herbal drugs over the modern medicine and very keen to conserve the Indian traditional use of herbal products, the current project is designed, choosing the medicinal plant against autism spectrum disorder, the neuro-developmental disorder, where no proper evidence has been registered in use of herbal medicine.

1.1. NEURODEVELOPMENTAL DISORDER: [10, 11]

Impairment growth and development of brain or central nervous system are known as neurodevelopment disorders. This term refers to a disorder of brain function that affects emotion, memory, self-control and ability of learning and that unfolds the individual grows. This term is exclusively used as a synonym of Inattention and Attention deficit hyperactivity disorder.

It is considered as neuro-developmental in origin and that has neuro- developmental consequence when occurs in infancy or childhood. This includes:

Inattention and Attention deficit hyperactivity disorder such as Asperger syndrome.

- Intellectual disability (ID) or intellectual and development disability (IDD).
- Fetal alcohol spectrum disorder.
- Traumatic brain injury including congenital injuries which causes cerebral plasy.[11]
- Motor disorders such as developmental coordination disorders, stereotypic movement disorder. Communication, speech and language disorder.
- Genetic disorder such as Fragile- X disorder.
- Down syndrome.
- Schizophrenia.

Neuro-developmental disorders are associated with widely varying degrees of difficulty which are significant mental, physical, emotional and economical consequence for individual and their family and society also.

1.2. CAUSE: [12, 13]

The development of brain is tightly regulated and genetically encoded process with clear influence from environment. This suggest that any deviation from this program early in life can result in neurodevelopment disorders and depending on specific timing, might lead to distinct pathology later in life. Because of that there are many causes of neurodevelopmental disorder, which can range from deprivation, genetic and metabolic diseases, immune disorder, infectious diseases, nutritional factor, physical trauma and environmental factors.

Some neurodevelopmental disorders are autism and other pervasive developmental disorders are considering multifactorial syndrome.

Various causes of neurodevelopment disorder:

- Immune disorder.
- Infectious disorder.
- Deprivation
- Genetic diseases.
- Metabolic diseases.

In early prenatal stage neurodevelopment start with a complex neurological development which begins with creation of neurons and radial glia and continue to develop in postnatal stage. This process will complete after 3yrs old.

Migration of neurons, which occurs from the second to the six month of gestation and again within the cerebellum postnatal, is a very important and complex process.

1.3 Children neurodevelopmental and intellectual disorder [14,15]

Neurodevelopmental Process

- Abnormalities in maturation may underlie neurodevelopmental disorder.

  Increased pruning with childhood –onset schizophrenia; decreased with autism 1 in 6 children in the industrialized countries: [16]

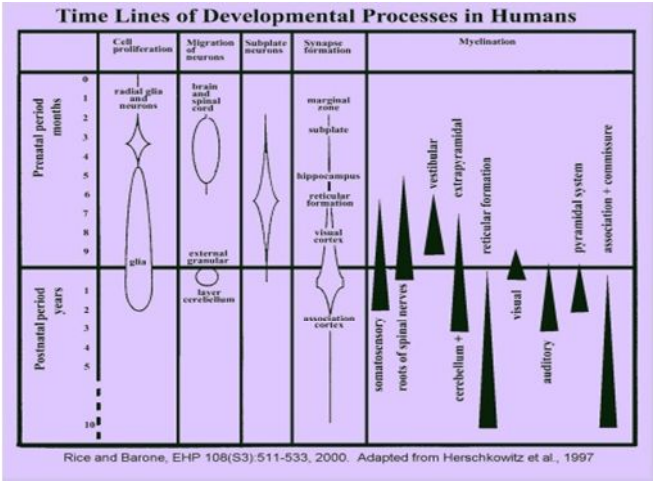


Fig.1. Neurodevelopment process in Human

- Cerebral palsy
- Autism
- Decreased IQ(intelligence quotient)
- ADHD (attention deficit hyperactivity disorder)
- Learning disabilities
- Developmental delay

In industrialized countries neuro-developmental disorders occurs commonly. It has been figured that 15% of children's are having learning disability, autism, ADHD, and developmental delay. The prevalence is much higher in abnormal children. Although some cases are linked to identify the exposure e.g.; fetal alcohol, tobacco smoke, low birth weight and in many cases etiology is not known.

2. REVIEW AND LITERATURE:

2.1 ATTENTION DEFICIT HYPERACTIVITY DISORDER:

ADHD is a neuro-developmental disorder and clinically heterogeneous disorder characterized by trio symptoms namely hyperactivity (excessive activity), inattention (problems in paying normal attention), and impulsivity (difficulty in having normal behavior or controlling behavior). ADHD is not just a childhood disorder it also occurs in adults. But most often the symptom of ADHD begins in childhood continues through adolescence and adulthood.

Some studies shows that hyperactivity improves as a child grows but the problems with inattention and impulsivity continue through the adolescence and into the adulthood.

Symptoms of ADHD usually occur when a person is below twelve to thirteen years of age and these symptoms should be present for at least six months or more than that. These symptoms create problems in day to day activities such as some tasks in schools or inability to grasp the theories or lectures or normal activities also interferes with the activities at home or recreational activities.

They (Symptoms) may also result in poor school performance. Some children may also find some task interesting due to symptoms of ADHD. Children with ADHD can be easily observed in home, clinics, and schools or at any other place. Due to inattention it causes distraction, daydreaming, difficulty in producing normal activity on a single task for prolonged period. Due to impulsivity it causes problems, disrupts classrooms, and causes misunderstanding with the peers. Due to hyperactivity it leads to excessive behavior than a normal person such as fidgeting, limitless talking which is difficult to tolerate in schools and they are also frustrating to guardians or protector or parents or ancestors, These peoples have difficulty in attaining normal sleep pattern,

Mostly adolescence with ADHD are at higher risk of drug abuse, nicotine dependence, low self-esteem, lack of confidence, mental struggle (or) conflict with parents, poor relationship with friends, family and people surrounding them.

2.1.1 DSM-IV, V: [17, 18]

It affects about 5–7% of children when diagnosed via the DSM-IV criteria and 1–2% when diagnosed via the ICD-10 criteria. As of 2015 it is estimated to affect about 51.1 million people.Rates are similar between countries and depend mostly on how it is diagnosed. ADHD is diagnosed approximately three times more often in boys than in girls, although the disorder is often overlooked in girls due to their symptoms differing from those of boys.About 30–50% of people diagnosed in childhood continue to have symptoms into adulthood and between 2–5% of adults have the condition.

According to American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders, Fifth edition: DSM-5 Washington: American Psychiatric Association, 2013.

Table no: 1

Place	Children Age (in years)	Percentage with ADHD	Year
U.S	2-5	2,37,000	2011 – 2012
U.S	2-5	↑50%	2007 – 2008
U.S	4-17	11%	2011 – 2012
U.S	4-17	↑7.8%	2003
U.S	4-17	↑9.5%	2007
U.S	4-17	11%	2011 – 2012
Nevada	4-17	5.6%	-
Kentucky	4-17	18.7%	-

2.2 HISTORY: [19]

The medical literature has described symptoms similar to ADHD since the 19th century. ADHD, its diagnosis, and its treatment have been considered controversial since the 1970s. The controversies have involved clinicians, teachers, policymakers, parents, and the media. Topics include ADHD's causes and the use of stimulant medications in its treatment. Most healthcare providers accept ADHD as a genuine disorder in children and adults, and the debate in the scientific community mainly centers on how it is diagnosed and treated. The

condition was officially known as **attention deficit disorder** (**ADD**) from 1980 to 1987 while before this it was known as **hyperkinetic reaction of childhood**. It was originally called hyperkinetic impulse disorder. It wasn't until the late 1960s that the American Psychiatric Association (APA) formally recognized ADHD as a mental disorder. Read more for a timeline of ADHD.

2.2.1 EARLY 1900s:

ADHD was first mentioned in 1902. British pediatrician Sir George Still described "an abnormal defect of moral control in children." He found that some affected children could not control their behavior the way a typical child would, but they were still intelligent.

2.2.2 THE INTRODUCTION OF BENZEDRINE:

The U.S. Food and Drug Administration (FDA) approved Benzedrine as a medicine in 1936. Dr. Charles Bradley stumbled across some unexpected side effects of this medicine the next year. Young patients' behavior and performance in school improved when he gave it to them. However, Bradley's contemporaries largely ignored his findings. Doctors and researchers began to recognize the benefit of what Bradley had discovered many years later.

2.2.3 NO RECOGNITION:

The APA issued the first "Diagnostic and Statistical Manual of Mental Disorders" (DSM) in 1952. This manual listed all of the recognized mental disorders. It also included known causes, risk factors, and treatments for each condition. Doctors still use an updated version today. The APA did not recognize ADHD in the first edition. A second DSM was published in 1968. This edition included hyperkinetic impulse disorder for the first time.

2.2.4 THE INTRODUCTION OF RITALIN:

The FDA approved the psychostimulant Ritalin (methylphenidate) in 1955. It became more popular as an ADHD treatment as the disorder became better understood and diagnoses increased. The medicine is still used to treat ADHD today.

2.2.5 A CHANGING DEFINITION:

The APA released a third edition of the DSM (DSM-III) in 1980. They changed the name of the disorder from hyperkinetic impulse disorder to attention deficit disorder (ADD). Scientists believed hyperactivity was not a common symptom of the disorder. This listing created two subtypes of ADD

- ADD with hyperactivity, and

- ADD without hyperactivity.

2.2.6 FINALLY, A NAME THAT FITS:

The APA released a revised version of the DSM-III in 1987. They removed the hyperactivity distinction and changed the name to attention deficit hyperactivity disorder (ADHD). The APA combined the three symptoms (inattentiveness, impulsivity, and hyperactivity) into a single type and did not identify subtypes of the disorder. The APA released the fourth edition of the DSM in 2000. The fourth edition established the three subtypes used by healthcare professionals today:

- combined type ADHD
- predominantly inattentive type ADHD
- predominantly hyperactive-impulsive type ADHD

2.3 CAUSES: [20]

Heredity is the most common cause of ADHD. Most of our information about the heritability of ADHD comes from family studies, adoption studies, twin studies and molecular genetic research.

2.3.1 FAMILY STUDIES:

If a trait has a genetic basis we would expect the rate of occurrence to be higher with the biological family members (e.g., brown-eyed people tend to have family members with brown eyes). Dr. Joseph Biederman (1990) and his colleagues at the Massachusetts General Hospital have studied families of children with ADHD. They have learned that ADHD runs in families. They found that over 25% of the first-degree relatives of the families of ADHD children also had ADHD, whereas this rate was only about 5% in each of the control groups. Therefore, if a child has ADHD there is a five-fold increase in the risk to other family members.

2.3.2 ADOPTION STUDIES:

If a trait is genetic, adopted children should resemble their biological relatives more closely than they do their adoptive relatives. Studies conducted by psychiatrist Dr. Dennis Cantwell compared adoptive children with hyperactivity to their adoptive and biological parents. Hyperactive children resembled their biological parents more than they did their adoptive parents with respect to hyperactivity.

2.3.3 TWIN STUDIES: [20]

Another way to determine if there is a genetic basis for a disorder is by studying large groups of identical and non-identical twins. Identical twins have the exact same genetic information while non-identical twins do not. Therefore, if a disorder is transmitted genetically, both identical twins should be affected in the same way and the concordance rate—the probability of them both being affected—should be higher than that found in non-identical twins. There have been several major twin studies in the past few years that provide strong evidence that ADHD is highly heritable. They have had remarkably consistent results in spite of the fact that they were done by different researchers in different parts of the world. In one such study, Dr. Florence Levy and her colleagues studied 1,938 families with twins and siblings in Australia. They found that ADHD has an exceptionally high heritability as compared to other behavioral disorders. They reported an 82 percent concordance rate for ADHD in identical twins as compared to a 38 percent concordance rate for ADHD in non-identical twins.

2.3.4 MOLECULAR GENETIC RESEARCH:

Twins studies support the hypothesis of the important contribution that genes play in causing ADHD, but these studies do not identify specific genes linked to the disorder. Genetic research in ADHD has taken off in the past five years. This research has focused on specific genes that may be involved in the transmission of ADHD. Dopamine genes have been the starting point for investigation. Two dopamine genes, DAT1 and DRD4 have been reported to be associated with ADHD by a number of scientists. Genetic studies revealed promising results, and we should look for more information about this soon. Typically, a number of genes are involved, many of which directly affect dopamine neurotransmission. Those involved with dopamine include DAT, DRD4, DRD5, TAAR1, MAOA, COMT, and DBH. Other genes associated with ADHD include SERT, HTR1B, SNAP25, GRIN2A, ADRA2A, TPH2, and BDNF. A common variant of a gene called LPHN3 is estimated to be responsible for about 9% of cases and when this variant is present, people are particularly responsive to stimulant medication. The 7 repeat variant of dopamine receptor D4 (DRD4–7R) causes increased inhibitory effects induced by dopamine and is associated with ADHD. The DRD4 receptor is a G protein-coupled receptor that inhibits adenylyl cyclase. The DRD4–7R

mutation results in a wide range of behavioral phenotypes, including ADHD symptoms reflecting split attention.

2.3.5 EXPOSURE TO TOXIC SUBSTANCES AS A CAUSE OF ADHD

Researchers have found an association between mothers who smoked tobacco products or used alcohol during their pregnancy and the development of behavior and learning problems in their children. A similar association between lead exposure and hyperactivity has been found, especially when the lead exposure occurs in the first three years. Nicotine, alcohol, and lead can be toxic to developing brain tissue and may have sustained effects on the behavior of the children exposed to these substances at early ages. However, it is unlikely that such exposure accounts for differences in brain development in the vast majority of children and adolescents with ADHD.

2.3.6 INJURY TO THE BRAIN FROM TRAUMA, BRAIN TUMORS, STROKES OR DISEASE

Injury to the brain can be the result of trauma (serious blow to the head), brain tumor, stroke or disease. These factors can cause problems with inattention and poor regulation of motor activity and impulses. While such circumstances can result in a diagnosis of ADHD, the occurrence of such is atypical.

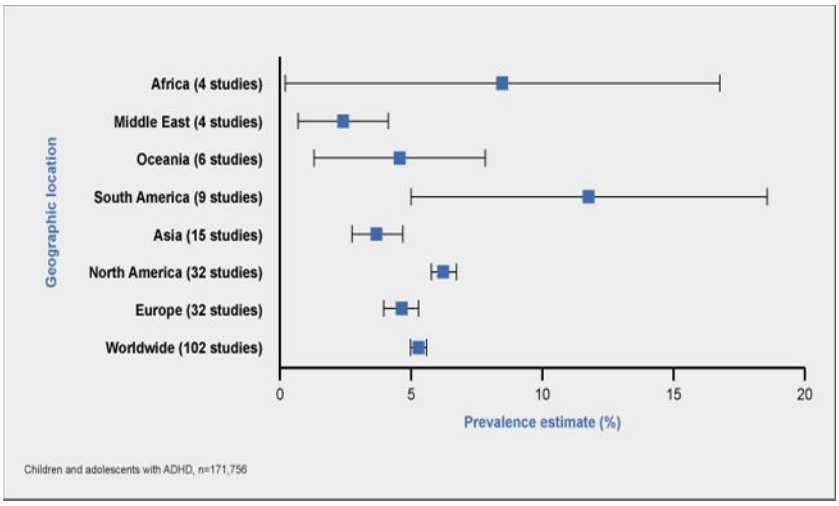
2.4 ADHD STATISTICS: [21]

Attention-deficit hyperactivity disorder (ADHD) or hyperkinetic disorder (HKD) affects people of all ages, and ADHD prevalence rates are known to vary between children, adolescents and adults.

Although there is no global consensus, meta-regression analyses have estimated the worldwide ADHD/HKD prevalence at between 5.29% and 7.1% in children and adolescents, and at 3.4% (range 1.2–7.3%) in adults.The prevalence of ADHD in very young children (aged <6 years) or later in adult life (aged >44 years) is less well studied.

A meta-analysis of studies (n=102) of children and adolescents diagnosed with ADHD found that the prevalence of ADHD in individuals aged ≤18 years varied between countries worldwide; the prevalence estimate for Europe specifically was just under 5% (Figure).

Fig no: 2



Geographical location was associated with significant variability between the prevalence estimates from North America and both the Middle East (p=0.01) and Africa (p=0.03), while no significant differences were reported for prevalence rates between North America and Europe (p=0.40), South America (p=0.83), Asia (p=0.85) or Oceania (p=0.45). This finding was confirmed in a meta-regression model using Europe as the comparator: significant differences in prevalence were found between Europe and both Africa (p=0.05) and the Middle East (p=0.03).

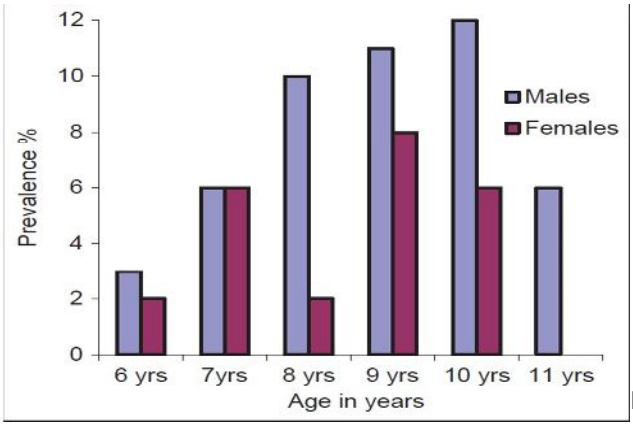
2.4.1 ADHD IN INDIA:

The prevalence rate of attention deficit hyperactivity disorder (ADHD), a condition almost always associated with poor academic performance, was 11.3% among primary school children; behavioral difficulties were found in 36.11% of the children with ADHD.

Although, research on A**DHD** in India suffers a severe lack of epidemiological studies, it is estimated that the prevalence figures may not vary much from the rest of the world.There is a huge gap between the needs and the services available for these children in India.

This is a cross sectional study of school aged children selected from four different schools in Coimbatore district.

Figure no 3: Distribution of ADHD among different age groups in males and females



[22]

2.5 SIGNS AND SYMPTOMS:

2.5.1 CLASSIFICATION OF ADHD BASED ON SYMPTOMS:

Three presentations of ADHD are defined in DSM-5™ based on the predominant symptom pattern for the past 6 months: [17]

- Combined presentation – all three core features are present and ADHD is diagnosed when ≥6 symptoms of hyperactivity/impulsivity and ≥6 symptoms of inattention have been observed for ≥6 months
- Predominantly inattentive presentation – diagnosed if ≥6 symptoms of inattention (but <6 symptoms of hyperactivity/impulsivity) have persisted for ≥6 months
- Predominantly hyperactive/impulsive presentation – diagnosed if ≥6 symptoms of hyperactivity/impulsivity (but <6 symptoms of inattention) have been present for ≥6 months.

2.5.2 MAJOR SYMPTOMS: [23]

- Inattention: getting distracted, having poor concentration and organizational skills
- Impulsivity: interrupting, taking risks
- Hyperactivity: never seeming to slow down, talking and fidgeting, difficulties staying on task

2.5.3 SYMPTOMS TYPES: [23]

2.5.3.1 TYPE 1

PREDOMINANTLY INATTENTIVE ADHD

If you have this type of ADHD, you may experience more symptoms of inattention than those of impulsivity and hyperactivity. You may struggle with impulse control or hyperactivity at times. But these aren't the main characteristics of inattentive ADHD.

People who experience inattentive behavior often:

- ✓ Miss details and are distracted easily
- ✓ Get bored quickly
- ✓ Have trouble focusing on a single task
- ✓ Have difficulty organizing thoughts and learning new information
- ✓ Lose pencils, papers, or other items needed to complete a task
- ✓ Don't seem to listen
- ✓ Move slowly and appear as if they're daydreaming
- ✓ Process information more slowly and less accurately than others
- ✓ Have trouble following directions
- ✓ More girls are diagnosed with inattentive type ADHD than boys.

2.5.3.2 TYPE 2:

PREDOMINANTLY HYPERACTIVE-IMPULSIVE ADHD:

This type of ADHD is characterized by symptoms of impulsivity and hyperactivity. People with this type can display signs of inattention, but it's not as marked as the other symptoms.

People who are impulsive or hyperactive often:

- ✓ Squirm, fidget, or feel restless
- ✓ Have difficulty sitting still
- ✓ Talk constantly
- ✓ Touch and play with objects, even when inappropriate to the task at hand
- ✓ Have trouble engaging in quiet activities
- ✓ Are constantly "on the go"
- ✓ Are impatient
- ✓ Act out of turn and don't think about consequences of actions

- ✓ Blurt out answers and inappropriate comments
- ✓ Children with hyperactive-impulsive type ADHD can be a disruption in the classroom. They can make learning more difficult for themselves and other students.

2.5.3.3 TYPE 3:

COMBINATION ADHD:

If you have the combination type, it means that your symptoms don't fit inattention or hyperactive-impulsive behavior.

- ✓ Most people, with or without ADHD, experience some degree of inattentive or impulsive behavior. But it's more severe in people with ADHD. The behavior occurs more often and interferes with how you function at home, school, work, and in social situations.

The National Institute of Mental Health explains that most children have combination type ADHD. The most common symptom in preschool-aged children is hyperactivity.

The current severity of ADHD should also be specified:

Mild – few, if any, symptoms in excess of those required to make the diagnosis are present, and symptoms result in no more than minor impairments in social or occupational functioning.

Moderate – symptoms or functional impairment between 'mild' and 'severe' are present.

Severe – many symptoms in excess of those required to make the diagnosis, or several symptoms that are particularly severe, are present; or the symptoms result in marked impairment in social or occupational functioning.

2.6 ADHD AND RELATED DISEASES OR CONDITIONS:[24]

ADHD doesn't increase a person's risk for other conditions or diseases. But some people with ADHD — especially children — are more likely to experience a range of co-existing conditions. They can sometimes make social situations more difficult or school more challenging.

Some co-existing conditions include:

- ✓ Learning disabilities
- ✓ Conduct disorders and difficulties, including antisocial behavior, fighting, and oppositional defiant disorder
- ✓ Anxiety disorder

- ✓ Depression
- ✓ Bipolar disorder
- ✓ Tourette's syndrome
- ✓ Substance abuse
- ✓ Bed-wetting problems
- ✓ Sleep disorders

2.7 DIAGNOSIS:[18]

ADHD is diagnosed by a sorting of a being's immature in activity and psychological exercise, including judgment out the effects of drugs, medications and additional scrutiny or psychiatric problems as explanations for the symptoms. It oftentimes takes into calculate feedback from parents and teachers with most diagnoses begun after a pedagogue raises concerns. It may be viewed as the extreme end of one or more unbroken anthropomorphic traits open in all fill. Whether someone responds to medications does not corroborate or restrict out the designation. As imagery studies of the brain do not afford conformable results between individuals, they are only old for research purposes and not designation.

In Northerly U.S.A., DSM-5 criteria are victimized for designation; spell Inhabitant countries commonly use the ICD-10. With the DSM-IV criteria a diagnosis of ADHD is 3-4 nowadays much liable than with the ICD-10 criteria. It is grouped as neurodevelopmental medicine change. Additionally, it is categorized as riotous activity disarray along with oppositional unwilling condition, carry disarray, and antisocial personality change. A diagnosing does not require a neurological disorder.

Related conditions that should be screened for countenance anxiety, depression, oppositional intractable disorder, direct upset and acquisition and faculty disorders. Remaining conditions that should be reasoned are added neurodevelopmental disorders, tics, and sleep apnea.

CHARACTERISTIC AND STATISTICAL DRILL:

As with umpteen additional medicine disorders, prescribed diagnosing should be prefabricated by a qualified athlete based on a set numerate of criteria. In the Federate States, these criteria are defined by the Dweller Medicine Relationship in the DSM. Based on the DSM criteria, there are triad sub-types of ADHD:

ADHD predominantly unalert typewrite (ADHD-PI) presents with symptoms including beingness easily distracted, forgetful, daydreaming, disturbance, penurious attention, and difficultness in completing tasks.

ADHD, predominantly hyperactive-impulsive type presents with undue restlessness and irritation, hyperactivity, sweat waiting and remaining sitting, embryonic doings; harmful behaviors may also be speak.

ADHD, one identify is a combining of the low two subtypes.

This segment is supported on presence of at minimal six out of nine long-term (long at small six months) symptoms of inattention, hyperactivity-impulsivity, or both. To be thoughtful, the symptoms staleness hump appeared by the age of six to seven and become in more than one environment (e.g. at home and at school time or activity). The symptoms staleness be not assume for a type of that age and there must be information that it is deed culture, edifice or operate akin problems.

There are two main classification systems for diagnosing ADHD:

2.7.1 THE INTERNATIONAL CLASSIFICATION OF MENTAL AND BEHAVIORAL DISORDERS 10TH REVISION (ICD-10):

The ICD-10 medical classification grouping refers to ADHD as hyperkinetic alter (HKD), a statue widely misused in Accumulation and included in Indweller clinical guidelines formulated with the Inhabitant Web for Hyperkinetic Disorders (EUNETHYDIS). This sorting scheme defines HKD as an unrelenting and nonindulgent impairment of psychological processing, characterized by "earliest onset; a combination of overactive, poorly softened activity with asterisked inattention and deficiency of unrelenting extend curiosity; and pervasiveness over situations and durability over clip of these behavioral characteristics".

2.7.2 THE AMERICAN PSYCHIATRIC ASSOCIATION'S DIAGNOSTIC AND STATISTICAL MANUAL OF MENTAL DISORDERS – 5TH EDITION (DSM-5^{TM})

The DSM-5TM scrutiny sorting scheme for ADHD is publicized by the Earth Psychiatric Relationship and is utilized in the USA and the break of the mankind. This categorization system defines ADHD as "a continual ornament of inattention and/or hyperactivity-impulsivity that interferes with process or usage".

DSM-5™ replaced the previous edition (DSM-IV) in 2013.

These medical classification systems may be used solo or in meeting with an array of rank scales, which often abstraction the scrap of ADHD upon author specialized areas of running or dimension of lifespan.

2.8 DETECTION TECHNIQUES:[25]

2.8.1 BRAIN FUNCTIONING AND FUNCTIONAL IMAGING TECHNIQUES

- Positron Emission Tomography (PET)
- Multichannel Electroencephalography (EEG)
- Magneto encephalography (MEG)
- Near Infrared Spectroscopic Imaging (NIRSI)
- Functional Magnetic Resonance Imaging (fMRI)

2.8.1.1 POSITRON EMISSION TOMOGRAPHY (PET)

In PET the mortal is honours injected with a make lived hot tracer isotope. The tracer is introduced into the body through a biologically live mote. After a ready period the hot molecules are intent in the desired paper and the message is settled low a scanner to book radioactive stuff of the tracer. In the impact of decay, the ammunition molecule produces an antilepton which in play generates two photons soaring in oppositeness directions. The detector can notice the photons to maneuver the locating of the emission. PET can detect the execution feed or glucose intake rates, which are the mealy mouthed measures of the brainpower state levels, by measuring the quantity of syndrome from a positioning. PET data has gymnasium spatial document (some 1-10 mm) at the expenditure of low temporal finding. For advance information delight refer to the credit

2.8.1.2 MULTICHANNEL ELECTROENCEPHALOGRAPHY (EEG)

The neurons communicate with each other by exchanging ionized particles through the synapses. The communication process constitutes the main part of the brain activity which causes an electrical current in the brain. EEG is a recording technique of brains electrical current for a short period of time. EEG can record the neuronal activity in a very high temporal frequency (in the range of milliseconds) but the spatial resolution is compromised.

2.8.1.3 MAGNETO ENCEPHALOGRAPHY (MEG)

The flow of ionized particles through neurons produces a weak magnetic field in the brain. MEG is a functional neuroimaging technique which can record the magnetic field produced by the electrical current due to neuronal activity. The brain activity level is then mapped with the recorded magnetic field. As the brain's magnetic field is very weak it is recorded using extremely sensitive magnetometers which use an array of superconducting quantum interference devices (SQUIDs). Similar to EEG it has very high temporal resolution and low spatial resolution.

2.8.1.4 NEAR INFRARED SPECTROSCOPIC IMAGING (NIRSI)

NIRSI is a non-invasive optical imaging technique which can be used as a functional brain imaging method. NIRSI uses near infrared (from about 800 nm to 2500 nm) electromagnetic signal to measure blood oxygenation changes in blood vessels of the brain by measuring the absorption of the near infrared signal emitted by the source onto the brain surface. The advantage of NIRSI is it is inexpensive, portable and can be used even when the subject is moving. NIRSI and functional Magnetic Resonance Imaging (fMRI) produce similar data as some previous studies have shown close spatial and temporal correlations when the data is recorded using the two methods.

2.8.1.5 FUNCTIONAL MAGNETIC RESONANCE IMAGING (FMRI)

As we used fMRI data for solving the ADHD classification problem, we provide the basic principles behind the data capturing method. The core concept of fMRI is based on the idea of the Nuclear Magnetic Resonance (NMR) technology which has been around for a long time.

NMR has a widespread application in the biomedical field for analyzing the characteristics of bio-molecules. The basic principles of NMR are explained in the next few sections without going into the mathematical details. The interested readers are referred to the following document for further details

2.9 PATHOPHYSIOLOGY OF ADHD: [26]

ADHD is noted for a trio of symptoms: inattention, hyperactivity, and impulsivity (figure 12-1). It is currently hypothesized that all these symptoms arise in part from abnormalities in various circuits involving the prefrontal cortex (figures 12-2 through 12-8). Specifically, the most prominent symptoms of inattention in ADHD, better known as

executive dys- function and as the inability to sustain attention and thus to solve problems, are hypothetically linked to inefficient information processing in the dorsolateral prefrontal cortex (dlpfc) (figures 12-2, 12-3, 12-7). Dlpfc is activated by a cognitive task known as the n-back test, which can be monitored in living patients doing it while in a functional magnetic resonance imaging (fmri) brain scanner (explained in figure 12-3).

Fig no: 4

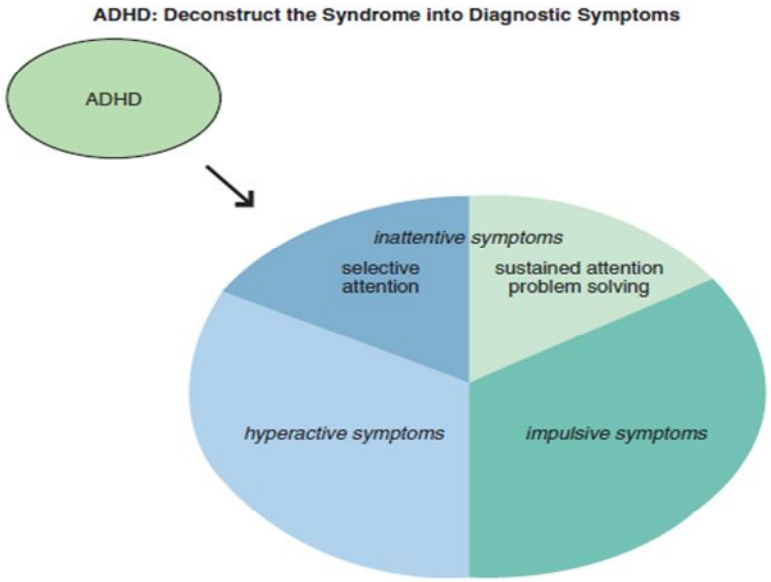


Figure 12-1. Symptoms of ADHD. There are three major categories of symptoms associated with attention deficit hyperactivity disorder (ADHD): inattention, hyperactivity, and impulsivity. Inattention itself can be divided into difficulty with selective attention and difficulty with sustained attention and problem solving.

Fig no: 5

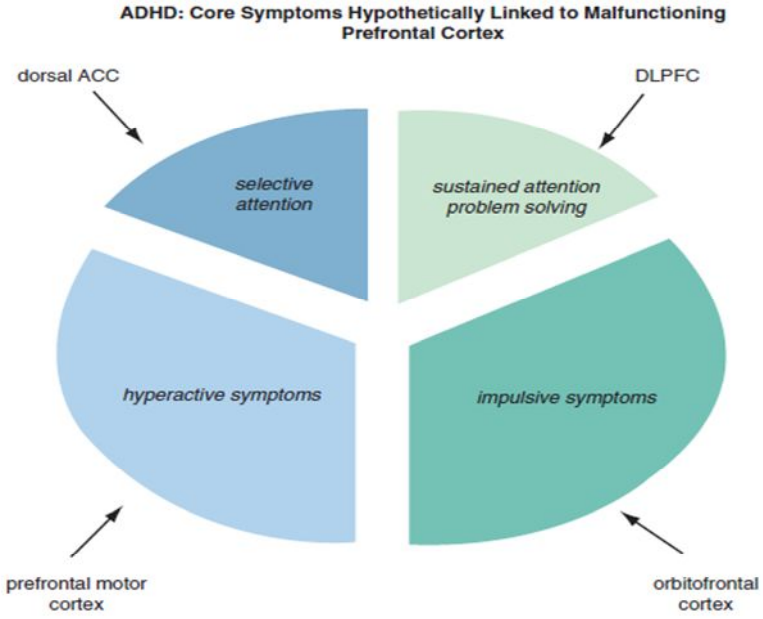


Figure 12-2. Matching ADHD symptoms to circuits. Problems with selective attention are believed to be linked to inefficient information processing in the dorsal anterior cingulate cortex (dACC), while problems with sustained attention are linked to inefficient information processing in the dorsolateral prefrontal cortex (DLPFC). Hyperactivity may be modulated by the prefrontal motor cortex and impulsivity by the orbitofrontal cortex (OFC).

Fig no: 6

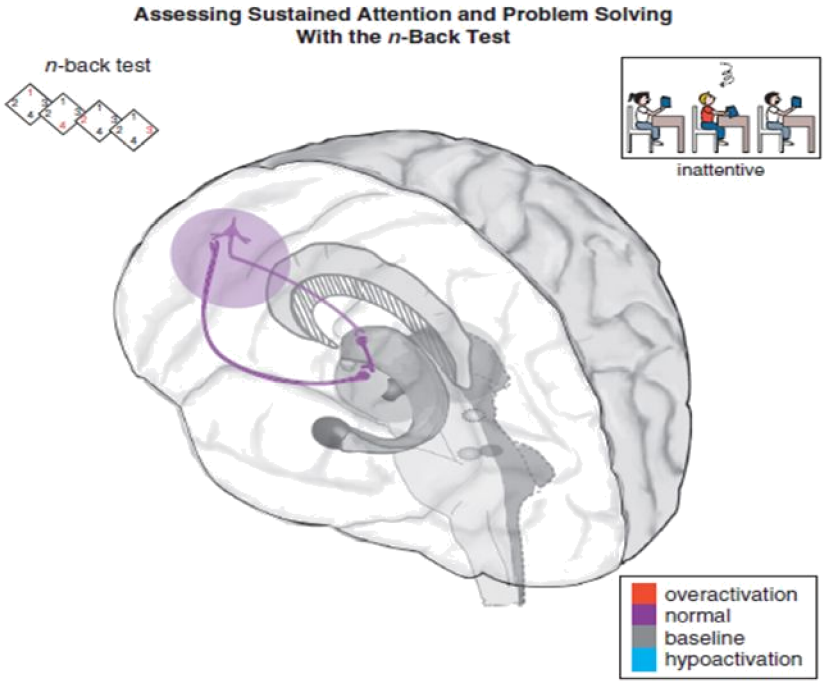


Figure 12-3. Sustained attention and problem solving: the *n*-back test. Sustained attention is hypothetically modulated by a cortico-striato-thalamo-cortical (CSTC) loop that involves the dorsolateral prefrontal cortex (DLPFC) projecting to the striatal complex. Inefficient activation of the DLPFC can lead to difficulty following through or finishing tasks, disorganization, and trouble sustaining mental effort. Tasks such as the *n*-back test are used to measure sustained attention and problem-solving abilities. In the 0-back variant of the *n*-back test, a participant looks at a number on the screen, and presses a button to indicate which number it is. In the 1-back variant, a participant only looks at the first number, and when the second number appears the participant is supposed to press a button corresponding to the first number. Higher *n* values are correlated with increased difficulty in the test.

Fig no: 7

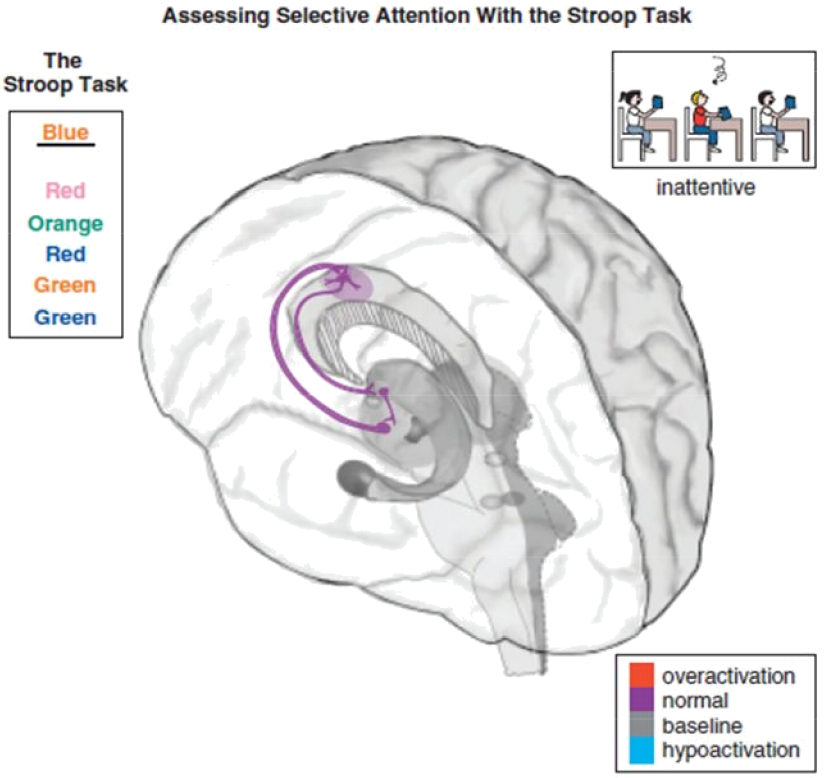


Figure 12-4. Selective attention: the Stroop task. Selective attention is hypothetically modulated by a cortico-striato-thalamo-cortical (CSTC) loop arising from the dorsal anterior cingulate cortex (dACC) and projecting to the striatal complex, then the thalamus, and back to the dACC. Inefficient activation of dACC can result in symptoms such as paying little attention to detail, making careless mistakes, not listening, losing things, being distracted, and forgetting things. An example of a test that involves selective attention, and thus should activate the dACC, is the Stroop task. The Stroop task requires the participants to name the color with which a word is written, instead of saying the word itself. In the present case, for example, the word "blue" is written in orange. The correct answer is therefore "orange," while "blue" is the incorrect choice.

Fig no: 8

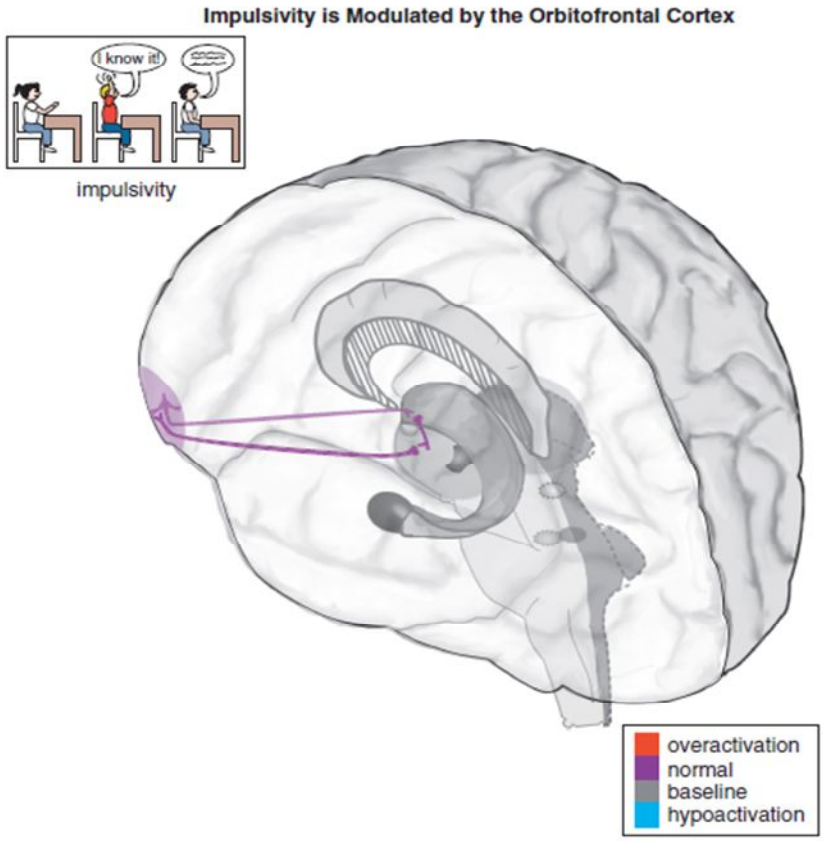


Figure 12-5. Impulsivity. Impulsivity is associated with a cortico-striato-thalamo-cortical (CSTC) loop that involves the orbitofrontal cortex (OFC), the striatal complex, and the thalamus. Examples of impulsive symptoms in ADHD include talking excessively, blurting things out, not waiting one's turn, and interrupting.

One can see how inefficient information processing in this particular DLPFC circuit when put under a cognitive "load" can be associated with the same symptom in many different psychiatric disorders. This is why diagnosis in psychiatry is now moving from describing categorical syndromes that mix together many symptoms (as in the DSM and ICD), towards characterizing single symptom domains such as executive dysfunction that cut across many psychiatric disorders, sometimes called Research Domain Criteria (RDC) for future diagnostic schemes that are set up to better correlate with neuroimaging and genetic findings.

Another symptom of ADHD is selective inattention, or not being able to focus, and thus differing from the executive dysfunction described above. The symptom of difficulty focusing is hypothetically linked to inefficient information processing in a different brain area, namely the dorsal anterior cingulate cortex (dACC) (Figures 12-2, 12-4, 12-7). The dACC can be activated by tests of selective attention, such as the Stroop test (explained in Figure 12-4). ADHD patients may either fail to activate the dACC when they should be focusing their

attention, or they activate this part of the brain very inefficiently and only with great effort and easy fatigability.

Other areas of prefrontal cortex that are hypothetically not functioning efficiently in ADHD are the orbitofrontal cortex (OFC), linked to symptoms of impulsivity (Figures 12-2, 12-5, 12-7) and the supplementary motor area, linked to symptoms of motor hyperactivity (Figures 12-2, 12-6, 12-7). The OFC is hypothetically linked to a wide variety of symptoms that cut across several psychiatric conditions, including impulsivity in ADHD (Figures 12-2, 12-5, 12-7), impulsivity and violence in schizophrenia, suicidality in depression, impulsivity in mania, and impulsivity/compulsivity in substance abuse. Impulsive symptoms in other psychiatric conditions commonly comorbid with ADHD are also hypothetically related to the orbitofrontal cortex, such as conduct disorder, oppositional defiant disorder, and bipolar disorder (Figure 12-8).

Fig no: 9

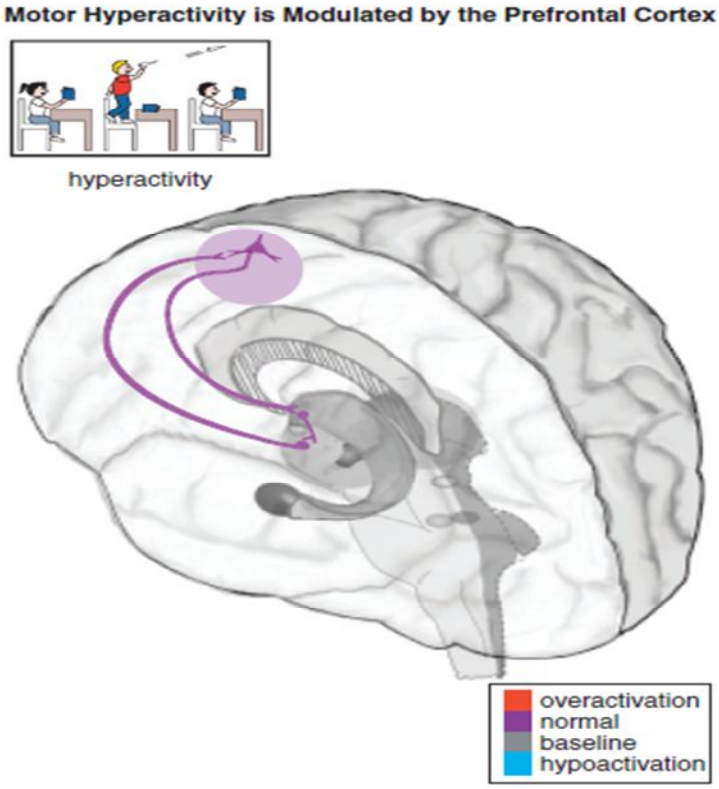


Figure 12-6. Hyperactivity. Motor activity, such as hyperactivity and psychomotor agitation or retardation, can be modulated by a cortico-striato-thalamo-cortical (CSTC) loop from the prefrontal motor cortex to the putamen (lateral striatum) to the thalamus and back to the prefrontal motor cortex. Common symptoms of hyperactivity in children with ADHD include fidgeting, leaving one's seat, running/climbing, being constantly on the go, and having trouble playing quietly.

ADHD as a disorder of inefficient "tuning" of the prefrontal cortex by dopamine and norepinephrine

ADHD patients generally cannot activate prefrontal cortex areas appropriately in response to cognitive tasks of attention and executive functioning. Some studies suggest that this is because dopamine (DA) and norepinephrine (NE) dysregulation in ADHD prevents the normal "tuning" of pyramidal neurons in the prefrontal cortex. In the case of DA and NE neurons, their normal firing at baseline is considered slow and "tonic," stimulating a few receptors on postsynaptic neurons and allowing for optimal signal transmission and downstream neuronal firing (Figure 12-9). Modest levels of NE release will hypothetically improve prefrontal cortical function by stimulating postsynaptic α2A receptors, but high levels of NE release will lead to impaired working memory when α1 and β1 receptors are also recruited (Figure 12-9). Similarly, modest levels of DA will first stimulate D3 receptors, as these are more sensitive to DA than D1 or D2 receptors (Figure 12-9). Hypothetically, low to moderate, but not high, levels of D1 receptor stimulation is beneficial to optimizing prefrontal cortical functioning.

Dopamine neurons in particular can also exhibit bursts of firing, called phasic (Figure 12-10). Phasic DA release is thought to reinforce learning and reward conditioning, providing the motivation to pursue naturally rewarding experiences such as education, recognition, career development, enriching social and family connections, etc. When the phasic DA system is hijacked by drugs, it can induce uncontrolled DA firing that reinforces the reward of drug abuse, and lead to compulsive behaviors such as mindless self-destructive drug. Thus, finely tuning the DA reward pathway in the nucleus accumbens and its connections to the amygdala and prefrontal cortex by attaining a low level of phasic firing in relation to tonic firing will theoretically lead to proper functioning of this complex system.

In ADHD, imbalances in NE and DA circuits in the prefrontal cortex hypothetically cause inefficient information processing in prefrontal circuits, and thus the symptoms of ADHD. At the level of NE and DA synapses in the prefrontal cortex, deficient signaling in prefrontal cortical DA and NE pathways is reflected by decreased neurotransmission and thus reduced stimulation of postsynaptic receptors (Figure 12-11). Agents that can lead to increased release of these two neurotransmitters or increased tonic firing of these neurons will be hypothetically beneficial in patients with ADHD by bringing pre- frontal activity back to

optimal levels. On the other hand, ADHD can also be hypothetically associated with excessive signaling in prefrontal cortical DA and NE pathways, particularly in adolescents and adults. That is, stress can activate NE and DA circuits in the prefrontal cortex, leading to high levels of DA and NE release, and thus cause an excess of phasic NE and DA firing. This excessive NE and DA neurotransmission may be the underpinning of the development of drug and alcohol abuse, impulsivity, inattention and anxiety, all comorbid with ADHD, particularly in adolescents and adults.

Fig no: 10

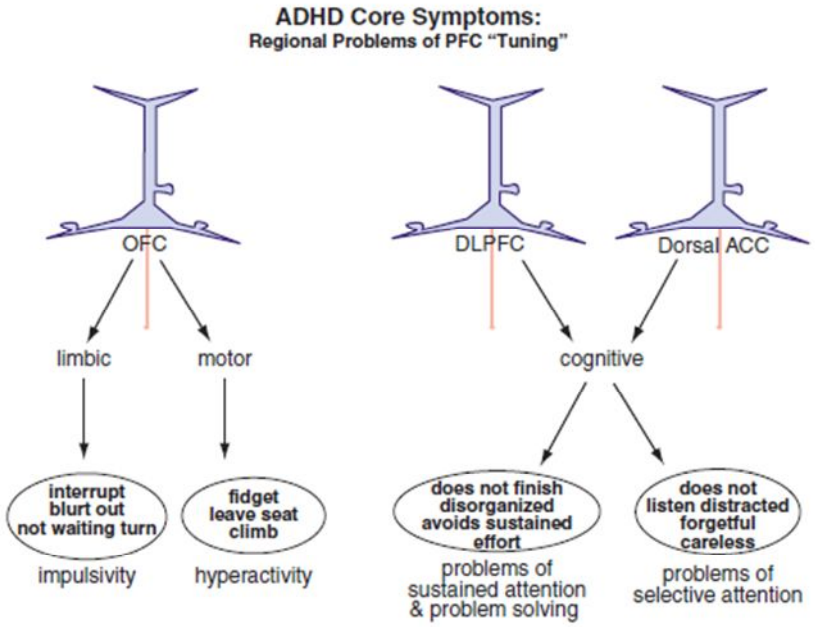


Figure 12-7. ADHD: out-of-tune prefrontal cortex. Different brain areas are hypothetically important in the symptoms of ADHD. Alterations within the orbitofrontal cortex (OFC) are hypothesized to lead to problems with impulsivity or hyperactivity. Inadequate tuning of the DLPFC or the dACC can respectively lead to sustained or selective attentive symptoms. It is becoming increasingly clear that dysfunction in specific brain areas leads to specific symptoms, such that abnormalities in the orbitofrontal–limbic motivation networks have been observed in children with conduct disorder, while aberrations in the dorsolateral cognitive network have been observed in children with problems of sustained attention.

Fig no: 11

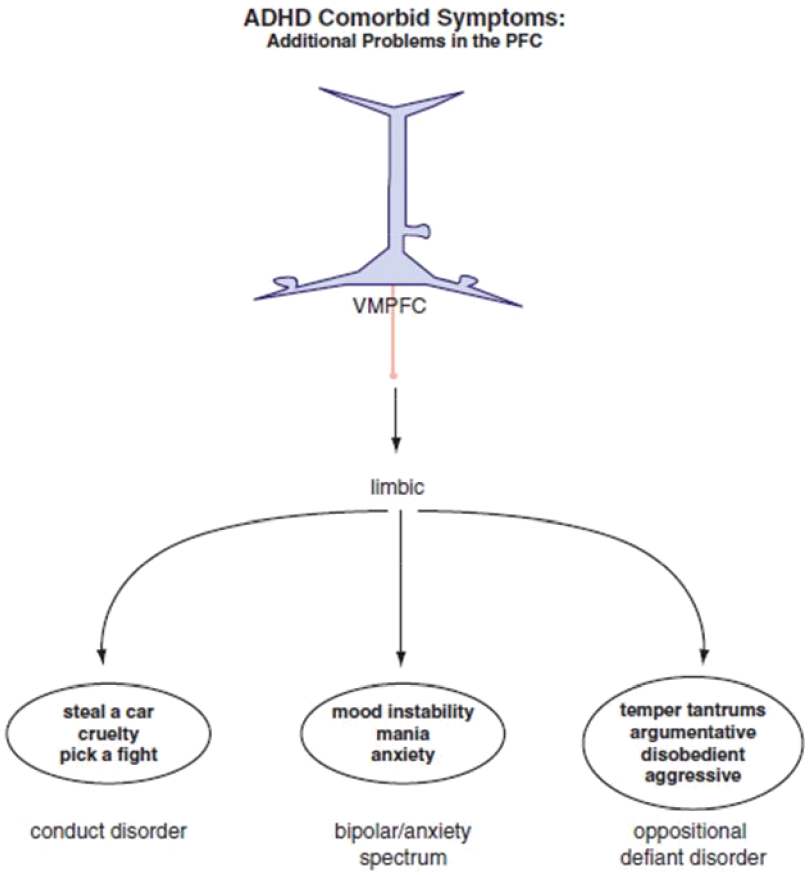


Figure 12-8. ADHD and comorbid symptoms. The comorbidities associated with ADHD are often the result of similar or additional dysfunctions within the prefrontal cortex–limbic network. Many mood disorders are comorbid with ADHD both in children and in adults, and it has been suggested that the symptoms in adults might be most disabling if the comorbidities were already present in the child. This emphasizes the importance of treating all the symptoms in the younger population of ADHD patients in order to maximize their chances of a "regular" adult life. VMPFC, ventromedial prefrontal cortex.

Fig no: 12

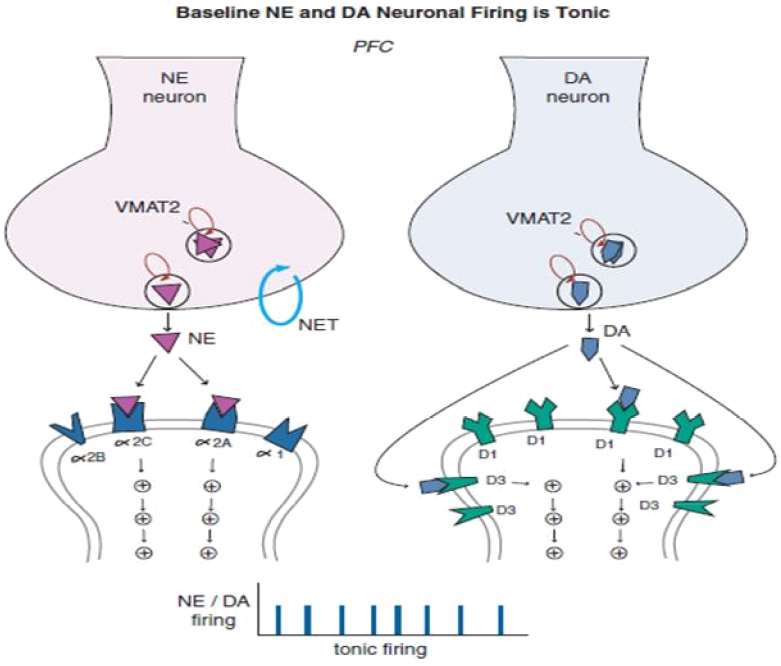


Figure 12-9. Baseline tonic firing. Modulation of prefrontal cortical function, and therefore regulation of attention and behavior, relies on the optimum release of dopamine (DA) and norepinephrine (NE). Under normal conditions, released NE and DA in the prefrontal cortex stimulate a few receptors on postsynaptic neurons allowing for optimal signal transmission and neuronal firing. At modest levels, NE can improve prefrontal cortical function by stimulating postsynaptic α_{2A} receptors, but will lead to impaired working memory at high levels when α_1 and β_1 receptors are also recruited. Similarly, modest levels of DA will first stimulate D_3 receptors as these are more sensitive to DA than D_1/D_2 receptors. Low to moderate, but not high, levels of D_1 receptor stimulation can be beneficial to prefrontal cortical functioning. In the case of both DA and NE systems, moderation is certainly key.

In the prefrontal cortex too much or too little stimulation by NE or DA can cause inefficient information processing, because for the prefrontal cortex to work properly, cortical pyramidal neurons need to be tuned, meaning that moderate stimulation of α2A receptors by NE and D1 receptors by DA is required, neither too high nor too low. In theory, the role of NE is to increase the incoming signal by allowing for increased connectivity of the prefrontal networks, while the role of DA is to decrease the noise by preventing inappropriate connections from taking place. Pyramidal cell function is optimal at the top of this inverted U-shaped curve, when stimulation of both α2A and D1 receptors is moderate. If stimulation at α2A and D1 receptors is too low, all incoming signals are the same, preventing a person from focusing on one single task (unguided attention). When stimulation is too high the signals get scrambled as additional receptors are recruited, again misguiding a person's attention. A balanced, moderate stimulation of α2A and D1 receptors is thus critical for correct interpretation of an incoming signal.

In prefrontal cortex, α2A and D1 receptors are often located on the spines of cortical pyramidal neurons, and can thus gate incoming signals. Alpha-2A receptors are linked to the molecule cyclic adenosine mono- phosphate (cAMP) via the inhibitory G protein, or Gi. D1 receptors, on the other hand, are linked to the cAMP signaling system via the stimulatory G protein (Gs). In either case, the cAMP molecule links the receptors to the hyperpolarization-activated cyclic nucleotide-gated (HCN) cation channels. An open channel will lead to a low membrane resistance, thus shunting inputs out of the spine. In the presence of an open channel, the signal leak's out and is therefore lost. However, when these channels are closed, the incoming signal survives and can be directed down the neuron to strengthen the network connectivity of similar neurons and lead to the appropriate signal and response.

When NE, or a noradrenergic agonist, binds to an α2A receptor, the activated Gi-linked system inhibits cAMP, thereby closing the HCN channel. Closure of the channel allows the signal to go through the spine and down the neuron, thereby strengthening network connectivity with similar neurons. So in general, in the prefrontal cortex, stimulation of α2A receptors strengthens an incoming signal.

By contrast, stimulation of D1 receptors leads to weakening of the signal. That is, when DA, or a DA agonist, binds to a D1 receptor, the activated Gs-linked system will lead to increased stimulation – or opening – of HCN channels. The opening of the HCN channels,

especially if excessive, will lead to leakage of the signal, thereby shunting any input out of the spine. So excessive stimulation of D1 receptors will, in contrast to stimulation of α2A receptors, result in the dissipation and/or weakening of a signal. The mechanism of action of α2A and D1 receptors explains in general why moderate stimulation of both types of receptors is preferred in order to strengthen the signal-to-noise ratio in prefrontal cortical neurons.

Fig no: 13

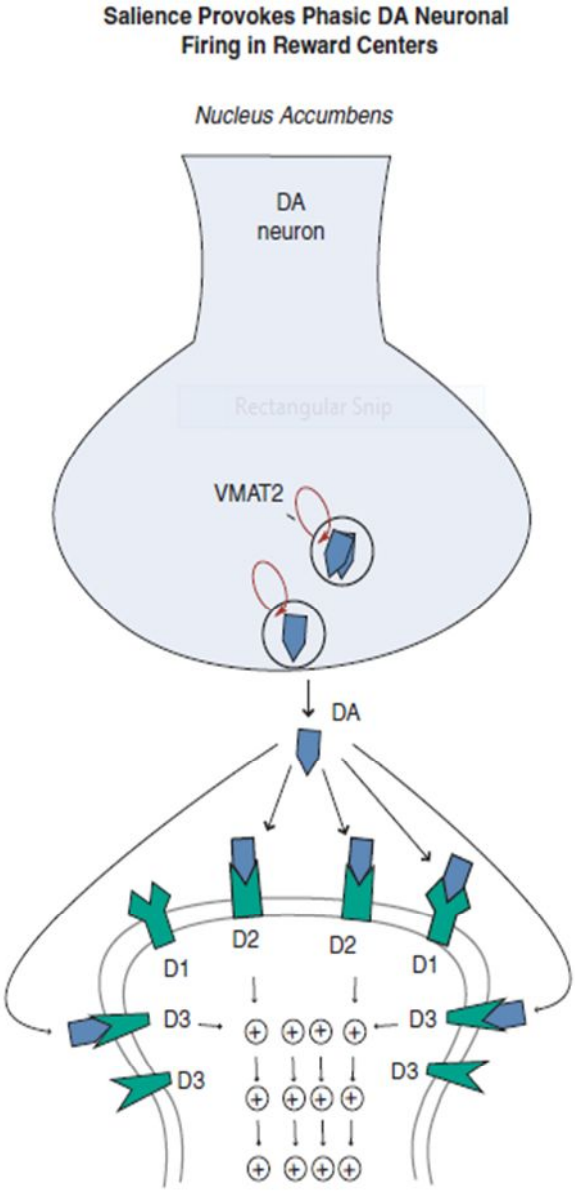


Fig no: 14

Figure 12-10. Salience-provoked phasic firing. While tonic firing, as seen in the prefrontal cortex, is often preferred in neuronal systems, a little bit of phasic firing of DA neurons in the nucleus accumbens can be a good thing. Phasic firing will lead to bursts of DA release, and when this happens in a controlled manner it can reinforce learning and reward conditioning, which can provide the motivation to pursue naturally rewarding experiences (e.g., education, career development, etc). When this system however is out of bounds, it can induce uncontrolled DA firing that reinforces the reward of taking drugs of abuse, for example, in which case the reward circuitry can be hijacked and impulses are followed by the development of uncontrolled compulsions to seek drugs.

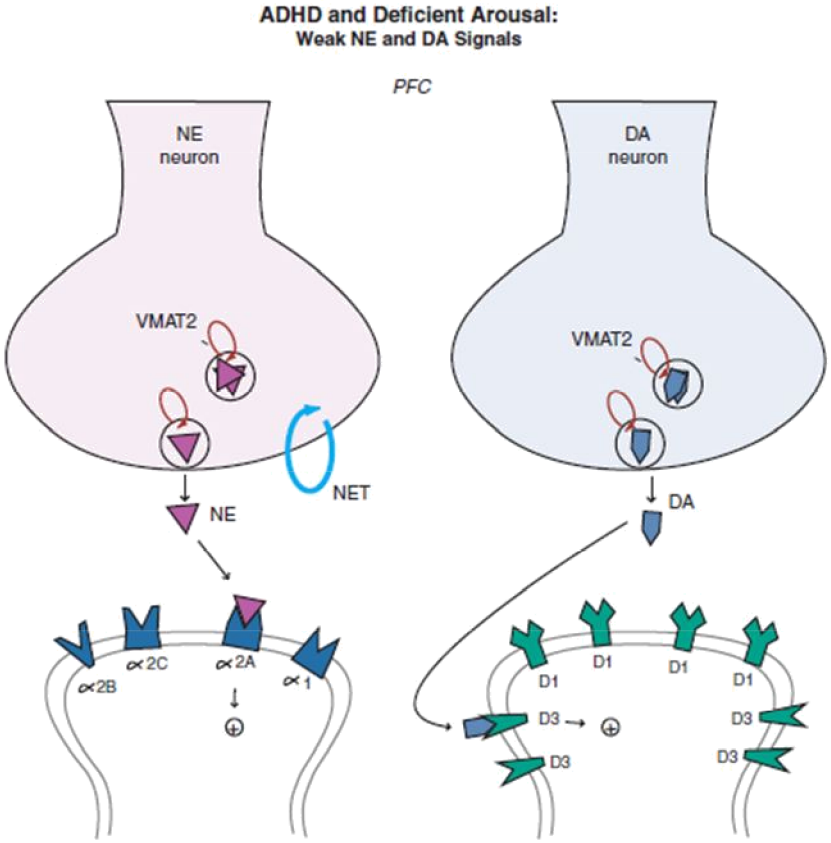


Figure 12-11. ADHD and deficient arousal. Besides being a key player in the arousal pathways, the prefrontal cortex is also the main brain area where imbalances in NE and DA systems hypothetically occur in ADHD. Deficient signaling in prefrontal cortical DA and NE pathways is reflected by reduced stimulation of postsynaptic receptors. Agents that can lead to (1) increased release of these two neurotransmitters, or (2) increased tonic firing of these neurons, will be hypothetically beneficial in patients with ADHD by bringing prefrontal activity back to optimal level.

The exact localization and density of α2A and D1 receptors within various cortical areas are still under intense investigation; it is possible to imagine the same pyramidal neuron receiving NE input from the locus coeruleus (LC) on one spine and DA input from the ventral tegmental area (VTA) on another spine. If the systems are properly "tuned," then D1 receptor stimulation can reduce the noise and α2A receptor stimulation can increase the signal to result

in proper prefrontal cortex functioning. Theoretically, this will result in adequate guided attention; focus on a specific task, and adequate control of emotions and impulses.

Deficient DA and NE input will theoretically lead to increased noise and decreased signal, respectively, thus preventing a coherent signal from being sent. Hypothetically, this could cause hyperactivity, inattention, impulsivity, or some combination of symptoms, depending upon the localization of the mistuned pyramidal neuron in prefrontal cortex (Figures 12-3 through 12-8). Furthermore, if one neurotransmitter is low while the other is high, then a person could be exhibiting a whole different set of symptoms. By knowing both the levels of DA and NE neurotransmission and the specific area of the possible disturbances, it may one day be possible to predict the degree and type of symptoms with which a patient is ailing. With this in mind, Figures 12-7 and 12-8 show how pyramidal neurons in different brain areas may be responsible for the different symptom presentations in ADHD.

2.9.1 BRAIN STRUCTURE:

In children with ADHD, there is a general reduction of volume in certain brain structures, with a proportionally greater decrease in the volume in the left-sided prefrontal cortex. The posterior parietal cortex also shows thinning in ADHD individuals compared to controls. Other brain structures in the prefrontal-striatal-cerebellar and prefrontal-striatal-thalamic circuits have also been found to differ between people with and without ADHD.

2.9.2 NEUROTRANSMITTER PATHWAYS:

Previously it was thought that the elevated number of dopamine transporters in people with ADHD was part of the pathophysiology but it appears that the elevated numbers are due to adaptation to exposure to stimulants. Current models involve the mesocorticolimbic dopamine pathway and the locus coeruleus-noradrenergic system. ADHD psychostimulants possess treatment efficacy because they increase neurotransmitter activity in these systems. There may additionally be abnormalities in serotoninergic, glutamatergic, or cholinergic pathways.

2.10 ADHD DRUG CLASSIFICATION: [27]

2.10.1 SHORT-ACTING:

Amphetamine/Dextroamphetamine (Adderall, Adderall XR)

Dextroamphetamine (Dexedrine, ProCentra, Zenzedi)

Dexmethylphendiate (Focalin)

Methylphenidate (Ritalin)

2.10.2 INTERMEDIATE-ACTING:

Amphetamine sulfate (Evekeo)

Methylphenidate (Ritalin SR, Metadate ER, Methylin ER)

2.10.3 LONG-ACTING:

Adzenys XR-ODT

Dexmethylphenidate (Focalin XR)

Dextroamphetamine (Adderall XR)

Lisdexamfetamine (Vyvanse)

Methylphenidate (Concerta, Daytrana, Metadate CD, Quillivant XR, Quillichew ER, Ritalin LA)

Mixed salts of a single-entity amphetamine product (Mydayis)

2.11 TREATMENT:

2.11.1 PHARMACOLOGICAL TREATMENT: [26]

Which symptoms should be treated priorly

It can be helpful in managing ADHD to prioritize which symptoms to target first with psychopharmacological treatments, even at the expense of delaying treatment for a while for some conditions, or even making some of these comorbid conditions transiently worse while other symptoms are targeted for improvement first. Although there are no definitive studies on this approach, clinical experience from many experts suggests that in such complex cases it can be very difficult to make any therapeutic progress if the patient continues to abuse alcohol or stimulants; thus substance-abuse problems must be managed top line. Treating ADHD may also have to await improvement from mood and anxiety disorder treatments, with ADHD seen as more of a fine-tune adjustment to a patient's symptom portfolio.

There are problems, however, with this approach of setting priorities of which symptoms and disorders to treat first. For example, many children are treated for their ADHD first and perhaps in isolation, without necessarily evaluating possible comorbidities until they fail to respond robustly to stimulant treatment. In adults it can be so difficult to treat substance abuse, mood disorders, and anxiety disorders that the focus of therapeutic attention never gets to ADHD or certainly to nicotine dependence. Once the mood or anxiety disorder is improving, treatment can plateau or stop. Too often the focus of psychopharmacological

management is the mood or anxiety disorder to the exclusion of any comorbid ADHD (or nicotine dependence). That is, ADHD can be considered a mere afterthought to be addressed if cognitive symptoms do not remit once the primary focus of therapeutic attention, namely the mood or anxiety disorder is treated. It is interesting that ADHD is not often the focus of treatment in adults unless it presents with no comorbid conditions. Since lack of comorbidity in adults with ADHD is rare, this may explain why the majority of adults with ADHD are not treated.

The modern, sophisticated psychopharmacologist keeps a high index of suspicion for the presence of ADHD in mood and anxiety and substance abuse disorders especially in adults, always aiming for complete symptomatic remission in patients under treatment. In practice, this means exploring the use of ADHD treatments as augmenting agents to first line treatments of mood, anxiety, and substance- abuse disorders, rather than the other way around. It also means for long-term management of ADHD to eventually address the treatment of nicotine dependence once the ADHD symptoms are under control. Adults with ADHD smoke as frequently as adults with schizophrenia, about twice the rate of the normal adult population in the US. This may be due to the fact that nicotine subjectively improves ADHD symptoms, especially in patients who are not treated for their ADHD. Nicotine enhances DA release and enhances arousal, so it is not surprising that it may be subjectively effective for ADHD symptoms.

2.11.1.1 STIMULANT TREATMENT OF ADHD

General principles

When both DA and NE are too low the strength of output in the prefrontal cortex is also too low, thus leading to reduced signal and increased noise (Figure 12-22A). Behaviorally, this could trans- late into a person not being able to sit in his/her seat and focus, and fidgeting and shifting attention (Figure 12-22A). In order to treat these symptoms, it is necessary to increase signal strength output by dialing up the release of both DA and NE until they reach the optimal levels (Figure 12-22B). This can be done both by stimulants and by some noradrenergic agents, as discussed below. Strengthening prefrontal cortical output is hypothesized to be beneficial in restoring a patient's ability to tease out important signals from unimportant ones, and to manage to sit still and focus.

Fig no: 15

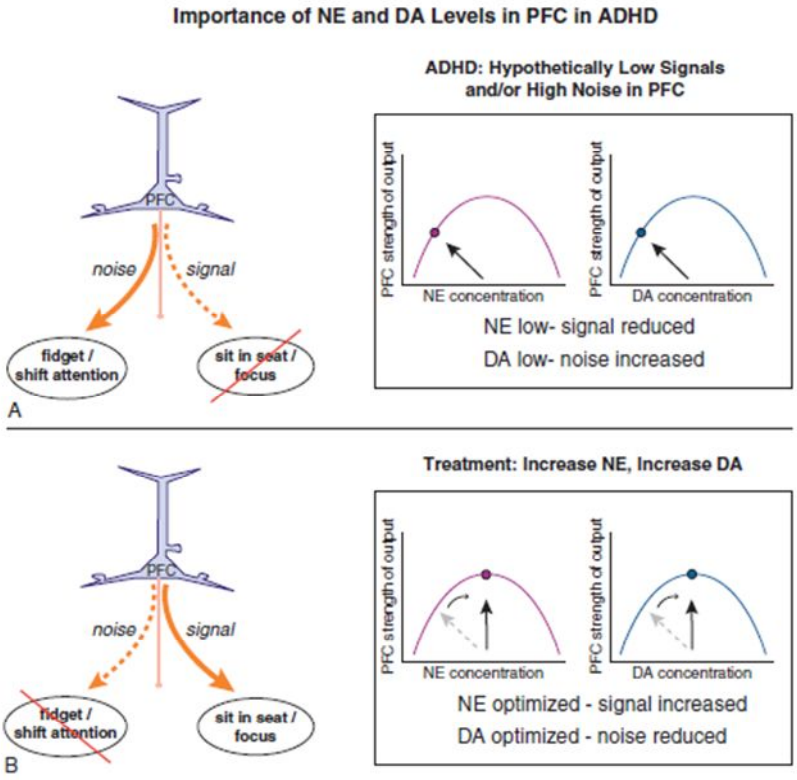


Figure 12-22. The importance of NE and DA levels in the PFC in ADHD. When both DA and NE are too low, i.e., on the left side of the inverted U-shaped curve, the strength of output in the prefrontal cortex is too low, leading to reduced signal and increased noise (A, right side). Inability to sit still and focus are often clinical manifestations of this imbalanced signal-to-noise ratio (A, left side). In order to treat these symptoms, it is necessary to increase strength output by dialing up (B, right side, toward the right on the U-shaped curve) the concentrations of both DA and NE until they reach the optimal dose (top of the inverted U-shaped curve).

Excessive as well as deficient activation of NE and DA in the prefrontal cortex can lead to ADHD as discussed above, namely by increasing the noise and decreasing the signal. The theory is that at first the added stress of suffering from ADHD, plus other stressors from the environment, can even further dial up the noise and reduce the signal, resulting in high NE and DA release, yet causing reduced signals and inefficient information processing. As stress becomes chronic, however, NE and DA levels eventually plummet due to depletion over time, but with no relief in terms of poor signal output. Ultimately the appropriate treatment is to increase NE and DA concentrations to allow for normalization of behavior.

Experienced clinicians are well aware that such patients with too much DA and NE, too little DA and NE, or a combination of these in different pathways, can be very difficult to treat. For example, in children, the combination of tics generally representing excessive DA activation in striatum may require DA-blocking antipsychotics and it can be very difficult to treat simultaneously in patients with ADHD who have deficient DA activation in cortex and require DA-enhancing stimulants. Stimulants may help the ADHD symptoms but make the tics much worse. Children and adolescents who have con- duct disorder, oppositional

disorders, psychotic disorders, and/or bipolar mania or mixed conditions comorbid with ADHD are among the most challenging patients for clinicians treating young patients.

Fig no: 16

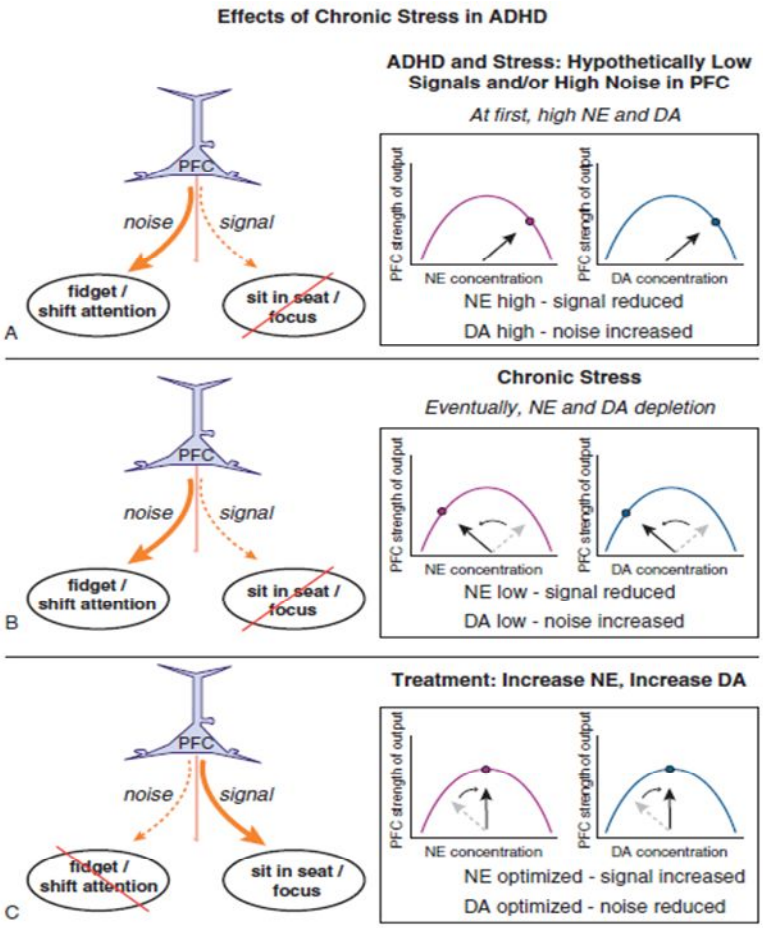


Figure 12-23. Chronic stress in ADHD. Excessive activation of NE and DA in prefrontal cortex (PFC) can lead to ADHD by increasing the noise and decreasing the signal. At first, the added stress of suffering from the disorder can further dial up the noise and reduce the signal (A: high NE and DA concentration leading to decreased output). As chronic stress sets in, NE and DA levels plummet (B: low NE and DA concentration also leading to decreased output), but with no relief in terms of signal output. Treatments that increase NE and DA concentrations may normalize behavior (C: noise is reduced and signal is increased).

Conditions of excessive DA activation suggest treatment with an atypical antipsychotic, yet ADHD suggests treatment with a stimulant. The rationale for this combination exploits the fact that atypical antipsychotics simultaneously release DA in prefrontal cortex to stimulate D1 receptors there while acting in limbic areas to block D2 receptors there. In patients who may require atypical antipsychotic treatment for psychotic or manic symptoms, yet still have ADHD, it is sometimes possible to augment the atypical antipsychotic cautiously with a stimulant, thereby increasing DA release to an even greater extent to act at D1 receptors in prefrontal cortex, hopefully reducing ADHD symptoms while blocking DA stimulation at D2 receptors sufficiently in limbic areas to prevent worsening of mania or psychosis. Such an approach is controversial and best left to experts for difficult patients who fail to improve adequately on monotherapies.

For adults with ADHD and anxiety, it can be difficult or even self-defeating to try to treat anxiety with SSRIs/ SNRIs or benzodiazepines while simultaneously administering a stimulant to improve the ADHD, only to cause the anxiety to worsen. For adults with ADHD and sub- stance abuse, it makes little sense to give stimulants to drug abusers in order to treat their ADHD. In these cases, augmenting antidepressant or anxiolytic therapies with a tonic activator of DA and/or NE systems such as a long-lasting NET inhibitor (norepinephrine reuptake inhibitors, NRIs), or an α2A-adrenergic agonist rather than a stimulant, can be an effective long-term approach for comorbid anxiety, depression, or substance abuse with ADHD. Some studies of NET inhibitors report improvement in both ADHD and anxiety symptoms, and other studies report improvement in both ADHD and heavy drinking. Further controlled trials are needed to clarify the responsiveness of both ADHD and comorbid conditions to treatment with NET inhibitors or α2A-adrenergic agonists.

METHYLPHENIDATE:

Fig no: 17

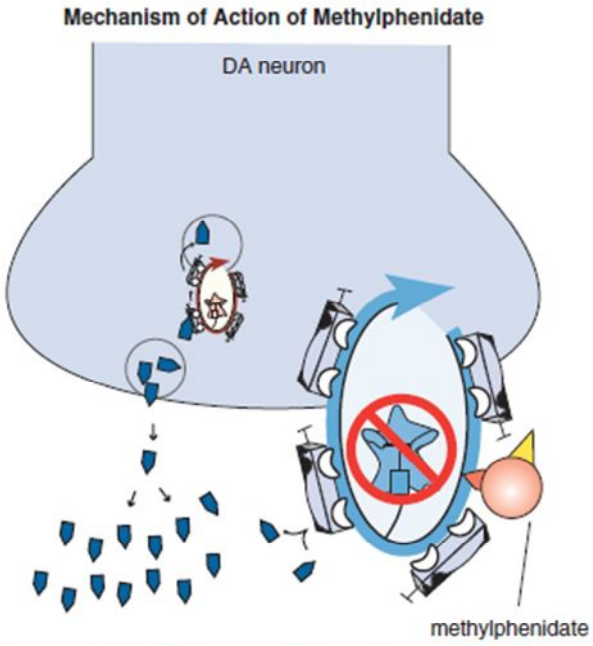


Figure 12-25B. Mechanism of action of methylphenidate: dopaminergic neurons. Methylphenidate works at DAT similar to how selective serotonin reuptake inhibitors (SSRIs) work at the serotonin transporter (SERT), namely by blocking the reuptake of DA into the terminal. Methylphenidate basically freezes the transporter in time, preventing DA reuptake and thus leading to increased synaptic availability of DA. Unlike amphetamine, methylphenidate is not itself taken up into the DA terminal via the transporter.

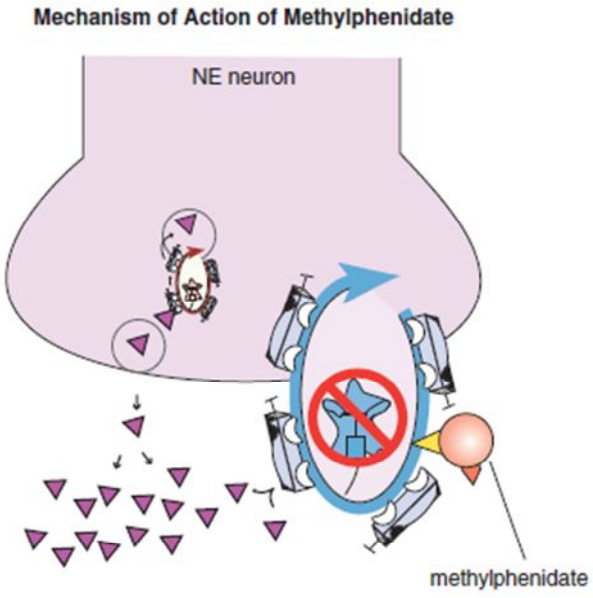


Figure 12-25C. Mechanism of action of methylphenidate: noradrenergic neurons. Methylphenidate works at NET in a manner similar to its actions at DAT, namely by blocking the reuptake of NE into the terminal. Methylphenidate freezes the transporter in time, preventing NE reuptake and thus leading to increased synaptic availability of NE. Unlike amphetamine, methylphenidate is not itself taken up into the NE terminal via the transporter.

Oral administration of clinically approved doses of the stimulant methylphenidate blocks the transporters for both NE and DA (NET and DAT).

Normally, dopamine is released, and then taken back up into the dopaminergic neuron by DAT, and finally stored in the synaptic vesicle by VMAT. Methylphenidate blocks DAT and NET allosterically, stopping the reuptake of dopamine via DAT (Figure 12-25B) and norepinephrine via NET (Figure 12-25C), with no actions on VMAT (Figures 12-25B and 12-25C). Methylphenidate blocks NET and DAT in much the same way as antidepressants block them, namely by binding to NET and DAT at sites distinct from where monoamines bind NET and DAT, i.e., allosterically. Thus, methylphenidate stops the reuptake pumps so that no methylphenidate is transported into the presynaptic neuron (Figures 12-25B and 12-25C). Methylphenidate has a d- and an l-isomer, with the d-isomer being much more potent than the l-isomer on both NET and DAT binding. Methylphenidate is available as the single enantiomer d-methylphenidate in both immediate-release and controlled-release preparations.

AMPHETAMINE:

Fig no: 18

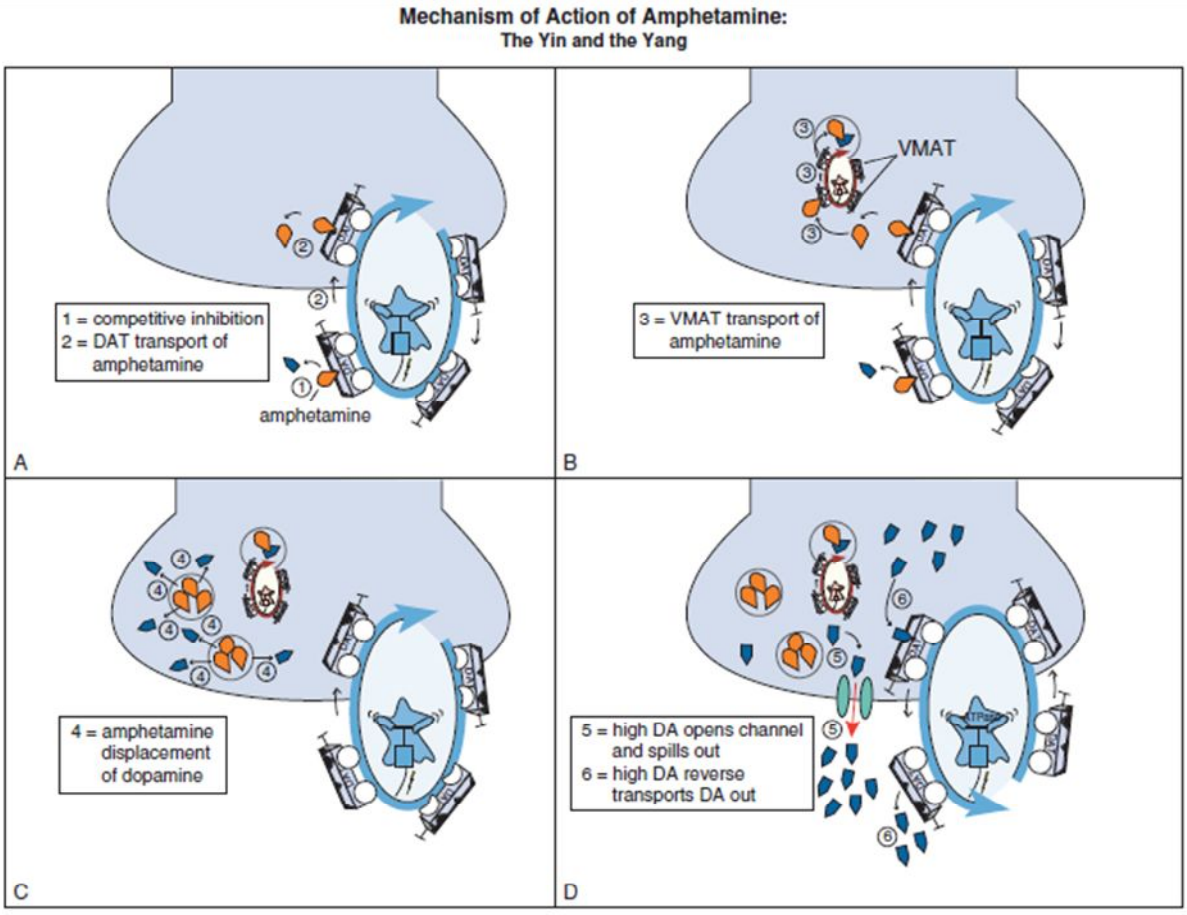


Figure 12-28. Mechanism of action of amphetamine: the yin and the yang. The yin – therapeutic and controlled drug delivery causes tonic-like increases; the yang – abusive doses and pulsatile drug delivery cause phasic-like increases. Shown here is amphetamine acting as a competitive inhibitor at DAT, thus competing with DA (1), or NE at NET (not shown). This is unlike methylphenidate's actions at DAT and NET, which are not competitive. Additionally, since amphetamine is also a competitive inhibitor of VMAT (a property that methylphenidate lacks) it is actually taken into the DA terminal via DAT (2), where it can then also be packaged into vesicles (3). At high levels, amphetamine will lead to the displacement of DA from the vesicles into the terminal (4). Furthermore, once a critical threshold of DA has been reached, DA will be expelled from the terminal via two mechanisms: the opening of channels to allow for a massive dumping of DA into the synapse (5) and the reversal of DAT (6). This fast release of DA will lead to the euphoric effect experienced after amphetamine use.

Oral administration of clinically approved doses of the stimulant amphetamine, like methylphenidate, also blocks the transporters both for NE and DA (NET and DAT), but in a different manner. Unlike methylphenidate and antidepressants, amphetamine is a competitive inhibitor and pseudosubstrate for NET and DAT (Figure 12-28), binding at the same site that the monoamines bind to the transporter, thus inhibiting NE and DA reuptake (Figure 12-28). At the doses of amphetamine used for the treatment of ADHD, the clinical differences in the actions of amphetamine versus methylphenidate can be relatively small. However, at the high

doses of amphetamine used by stimulant addicts, additional pharmacologic actions of amphetamine are triggered. Following competitive inhibition of DAT amphetamine is actually transported as a hitchhiker into the presynaptic DA terminal, an action not shared by methylphenidate or antidepressants. Once there in sufficient quantities, such as occurs with doses taken for abuse, amphetamine is also a competitive inhibitor of the vesicular transporter (VMAT2) for both DA and NE. Once amphetamine hitchhiker another ride into synaptic vesicles, it displaces DA there, causing a flood of DA release. As DA accumulates in the cytoplasm of the presynaptic neuron, it causes the DAT to reverse directions, spilling intracellular DA into the synapse, and also opening presynaptic channels to further release DA in a flood into the synapse. These pharmacologic actions of high-dose amphetamine are not linked to any therapeutic action in ADHD but to reinforcement, reward, euphoria, and continuing abuse. Amphetamine has a d- and an l-isomer. The d-isomer is more potent than the l-isomer for DAT binding, but d- and l-amphetamine isomers are more equally potent in their actions on NET binding. Thus, d-amphetamine preparations will have relatively more action on DAT than NET; mixed salts of both d- and l-amphetamine will have relatively more action on NET than d-amphetamine but overall still more action on DAT than NET. These pharmacological mechanisms of action of the stimulants come into play particularly at lower therapeutic doses utilized for the treatment of ADHD. D-Amphetamine also comes in a formulation linked to the amino acid lysine which is not absorbed until slowly cleaved into active d-amphetamine in the stomach, and slowly, rather than rapidly, absorbed.

SLOW-RELEASE VERSUS FAST-RELEASE STIMULANTS AND THE MYSTERIOUS DAT:

Rapid and high degrees of DAT occupancy by stimulants may cause euphoria and lead to abuse, whereas slow onset and lower degrees of DAT occupancy may be consistent with antidepressant actions and improvement in attention in ADHD. The DAT appears therefore to be a somewhat mysterious target for drugs, giving one set of responses if occupancy by a given stimulant is rapid, saturating, and short-acting (namely, resulting in “highs” and reinforcement and eventually compulsive use) and a completely different set of responses if occupancy by that same stimulant of that very same DAT target ramps up slowly, has incomplete target saturation, and lasts a long time (resulting in therapeutic actions in ADHD and depression without “highs” or abuse). Thus, pharmacokinetic

considerations seem to be just as important to the actions of stimulants in general, and in ADHD in particular, as their pharmacodynamic mechanisms.

Clinicians, parents, and patients often ask if there is a difference between the use of stimulants in the treatment of ADHD and the abuse of stimulants in substance-use disorders. The difference lies less in the mechanism of action, but more in the nature of the mysterious DAT, which has very different clinical responses to different routes of administration and doses, and thus how quickly, how strongly, and how completely DAT is blocked. When using stimulants to treat a patient it may be preferable to obtain a slow-rising, constant, steady-state level of the drug. Under those circumstances the firing pattern of DA will be tonic, regular, and not at the mercy of fluctuating levels of DA. Some pulsatile firing is fine, especially when involved in reinforcing learning and salience. However, DA stimulation follows an inverted U-shaped curve, such that too much DA will mimic the actions of DA in stress at higher doses, or mimic drug abuse at the highest doses. Thus a pulsatile drug administration that causes intermittent release of DA, unlike constant release, will lead to the highly reinforcing pleasurable effects of drugs of abuse.

The past several years have seen a flurry of new drug development activities aimed at optimizing the drug delivery characteristics of stimulants for ADHD. These are not mere patent extension gimmicks, nor mere convenience features, although it is certainly an advantage for a child not to have to take a second dose of a stimulant in the middle of the day at school. More importantly, the “slow-dose” stimulants optimize the rate, the amount, and the length of time that a stimulant occupies NET and DAT for therapeutic use in ADHD. Optimization for ADHD means occupying enough of the NET in prefrontal cortex at a slow enough onset and long enough duration of action to enhance tonic NE signaling there via α2A receptors and to increase tonic DA signaling there via D1 receptors, yet occupying little enough of the DAT in nucleus accumbens so as not to increase phasic signaling there via D2 receptors. It appears that ADHD patients have their therapeutic improvement by stimulants at the mercy of how fast, how much, and for how long stimulants occupy NET and DAT. When this is done in an ideal manner with slow onset, robust but sub- saturating drug levels, and long duration of action before declining and wearing off, the patient benefits with improved ADHD symptoms, hours of relief, and no euphoria. Tonic drug delivery of stimulants amplifies the desired tonic increases in DA and NE action for ADHD improvement for

several hours.

2.11.1.2 NORADRENERGIC TREATMENT OF ADHD ATOMOXETINE:

Fig no: 19

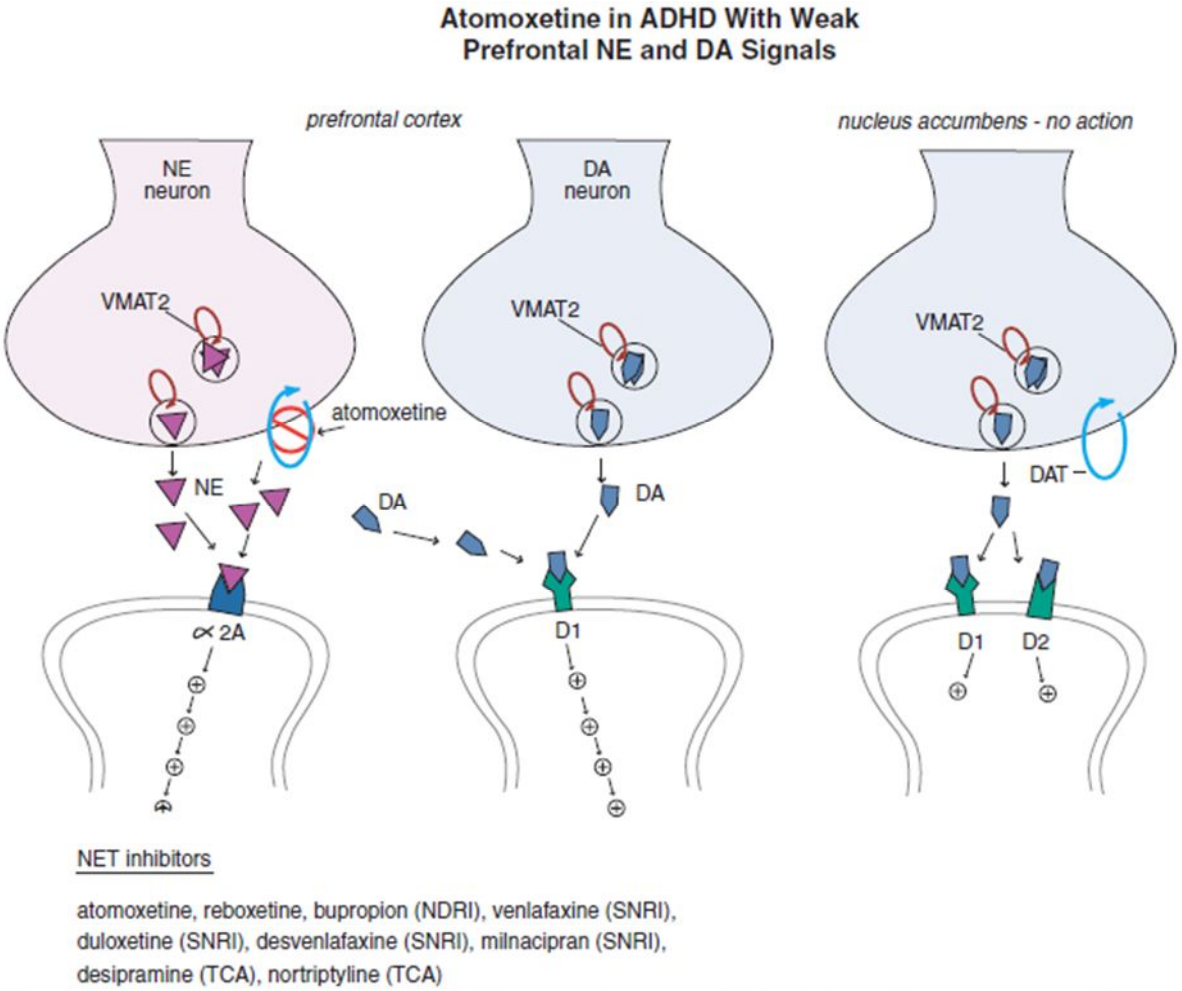


Figure 12-32. Atomoxetine in ADHD with weak prefrontal norepinephrine and dopamine signals. It has been suggested that atomoxetine can have therapeutic effects in ADHD without abuse potential. As a norepinephrine reuptake blocker, atomoxetine causes NE and DA levels to increase in the prefrontal cortex, where inactivation of both of these neurotransmitters is largely due to NET (on the left). At the same time, the relative lack of NETs in the nucleus accumbens prevents atomoxetine from increasing NE or DA levels in that brain area, thus reducing the risk of abuse (on the right). Thus, as shown in Figure 12-22, by increasing NE and DA levels to their optimal levels in the prefrontal cortex (top of the inverted U-shaped curve), atomoxetine may be able to increase attention and decrease hyperactivity in patients with ADHD.

Atomoxetine is a selective norepinephrine reuptake inhibitor or selective NRI. Sometimes called NET inhibitors, the selective NRIs have known antidepressant properties. In terms of their mechanism of therapeutic action in ADHD, since the prefrontal cortex lacks high concentrations of DAT, DA is inactivated in this part of the brain by NET. Thus, inhibiting NET increases both DA and NE in prefrontal cortex. However, since there are few

NE neurons and NETs in nucleus accumbens, inhibiting NET does not lead to an increase in either NE or DA there (Figure 12-32). For this reason, in ADHD patients with weak NE and DA signals in prefrontal cortex, a selective NRI such as atomoxetine increases both NE and DA in prefrontal cortex, enhancing tonic signaling of both, but increases neither NE nor DA in accumbens. Therefore, atomoxetine has no abuse potential.

Atomoxetine is the only such agent approved for use in ADHD, but several other agents have NRI actions, including the approved (outside of the US) antidepressant and selective NRI reboxetine, and the various SNRIs, which not only have NRI actions but also serotonin reuptake inhibiting properties.

Bupropion is a weak NRI and also a weak DAT inhibitor known as a norepinephrine–dopamine reuptake inhibitor (NDRI). Several tricyclic antidepressants have notable NRI actions, such as desipramine and nortriptyline. All of these agents with NRI properties have been utilized in the treatment of ADHD, with varying amounts of success, but only atomoxetine is well investigated and approved for this use in children and adults.

Atomoxetine's hypothetical actions in ADHD patients with stress and comorbidity states presumably linked to excessive and phasic DA and NE release are shown conceptually by comparing the untreated states with the changes that theoretically follow chronic treatment with atomoxetine. That is, ADHD linked to conditions that are associated with chronic stress and comorbidities is theoretically caused by overly active NE and DA circuits in prefrontal cortex causing an excess of phasic NE and DA activity. When slow-onset, long-duration, and essentially per-petual NET inhibition occurs in prefrontal cortex due to atomoxetine, this theoretically restores tonic post- synaptic D1 and α2A-adrenergic signaling, down regulates phasic NE and DA actions, and desensitizes postsynaptic NE and DA receptors. The possible con- sequences of this are to reduce chronic HPA axis overactivation and thereby potentially reverse stress- related brain atrophy and even induce neurogenesis that could protect the brain. Such biochemical and molecular changes could be associated with decreases in ADHD symptoms, reduction of relapse, and decreases in anxiety, depression, and heavy drinking. Unlike stimulant use, where the therapeutic actions are at the mercy of plasma drug levels and momentary NET/DAT occupancies, actions from long-term NRI actions give 24-hour symptom relief, in much the same manner as do SSRIs and SNRIs for the treatment of depression and anxiety. Such possibilities are already

indicated by early clinical investigations of this mechanism of selective NRI action in ADHD, but much further work is necessary to establish with certainty the long- term effects of selective NRI action, the differences of outcomes if any compared to long-term stimulant actions, and the best ADHD patient profile to choose for the selective NRI mechanism. Selective NRIs generally have smaller effect sizes for reducing ADHD symptoms than stimulants in short-term trials, especially in patients without comorbidity. However, NRIs are not necessarily inferior in ADHD patients who have not been previously treated with stimulants, or in ADHD patients who have been treated long-term (longer than 8–12 weeks). NRIs may actually be preferred to stimulants in patients with complex comorbidities.

ALPHA-2A-ADRENERGIC AGONISTS:

There are numerous subtypes of α-adrenergic receptors, from presynaptic autoreceptors, generally of the α2A subtype to postsynaptic α2A, α2B, α2C, and α1 subtypes. Alpha-2A receptors are widely distributed throughout the CNS, with high levels in the cortex and locus coeruleus. These receptors are thought to be the primary mediators of the effects of NE in prefrontal cortex regulating symptoms of inattention, hyperactivity, and impulsivity in ADHD. Alpha-2B receptors are in high concentrations in the thalamus and may be important in mediating sedating actions of NE, while α2C receptors are densest in striatum. Alpha-1 receptors generally have opposing actions to α2 receptors, with α2 mechanisms predominating when NE release is low or moderate (i.e., for normal attention), but with α1 mechanisms predominating at NE synapses when NE release is high (e.g., associated with stress and comorbidity) and contributing to cognitive impairment. Thus, selective NRIs at low doses will first increase activity at α2A postsynaptic receptors to enhance cognitive performance, but at high doses may swamp the synapse with too much NE and cause sedation, cognitive impairment or both. Patients with these responses to selective NRIs may benefit from lowering the dose. Alpha-2-adrenergic receptors are present in high concentrations in the prefrontal cortex, but only in low concentrations in the nucleus accumbens.

There are two direct-acting agonists for α2 receptors used to treat ADHD, guanfacine and clonidine. Guanfacine is relatively more selective for α2A receptors. Recently, guanfacine has been formulated into a controlled-release product, guanfacine ER that allows once-daily administration, and lower peak- dose side effects than immediate-release

guanfacine. Only the controlled-release version of guanfacine is approved for treatment of ADHD.

Clonidine is a relatively nonselective agonist at α2 receptors, with actions on α2A, α2B, and α2C receptors. In addition, clonidine has actions on imidazoline receptors, thought to be responsible for some of clonidine's sedating and hypotensive actions. Although the actions of clonidine at α2A receptors exhibit therapeutic potential for ADHD, its actions at other receptors may increase side effects. Clonidine is approved for the treatment of hypertension, but not for the treatment of ADHD, conduct disorder, oppositional defiant disorder, or Tourette's syndrome for which it is often used "off-label."

By contrast, the selective α2A receptor agonist guanfacine is 15–60 times more selective for α2A receptors than for α2B and α2C receptors. Additionally, guanfacine is 10 times weaker than clonidine at inducing sedation and lowering blood pressure, yet it is 25 times more potent in enhancing prefrontal cortical function. Thus, it can be said that guanfacine exhibits therapeutic efficacy with a reduced side- effect profile compared to clonidine. The therapeutic benefits of guanfacine are related to the direct effects of the drug on postsynaptic receptors in the PFC, which lead to the strengthening of network inputs, and to behavioral improvements

Fig no 20:

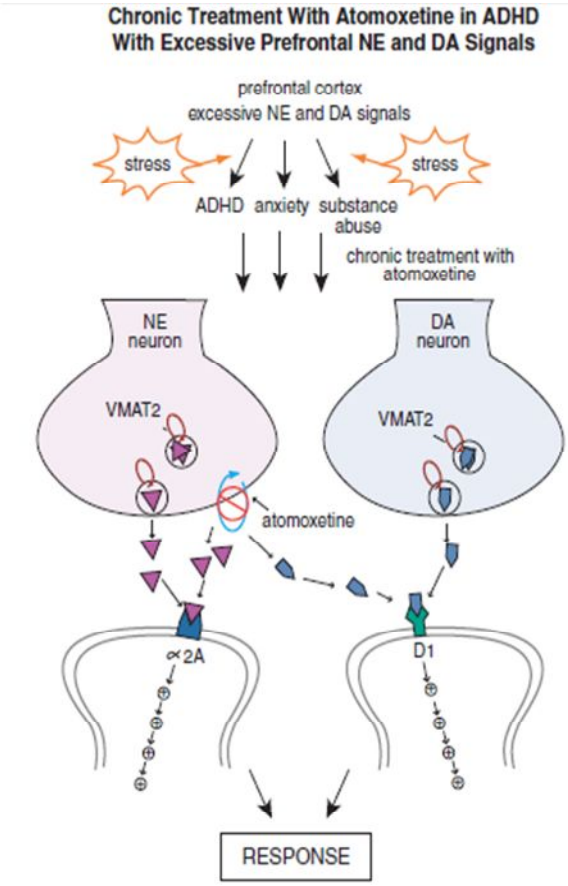


Figure 12-33. Chronic treatment with atomoxetine in ADHD. Stress combined with excessive NE and DA signaling can lead to ADHD, anxiety, or substance abuse. One way to reduce excessive stimulation could be to desensitize postsynaptic DA and NE receptors, and thus allow the neurons to return to normal tonic firing over time. By continuously blocking NET, atomoxetine has the capability of doing this. The "big picture" ramification of such a treatment could be decreased anxiety, decreased heavy drinking, and a reduction in relapses of substance abuse.

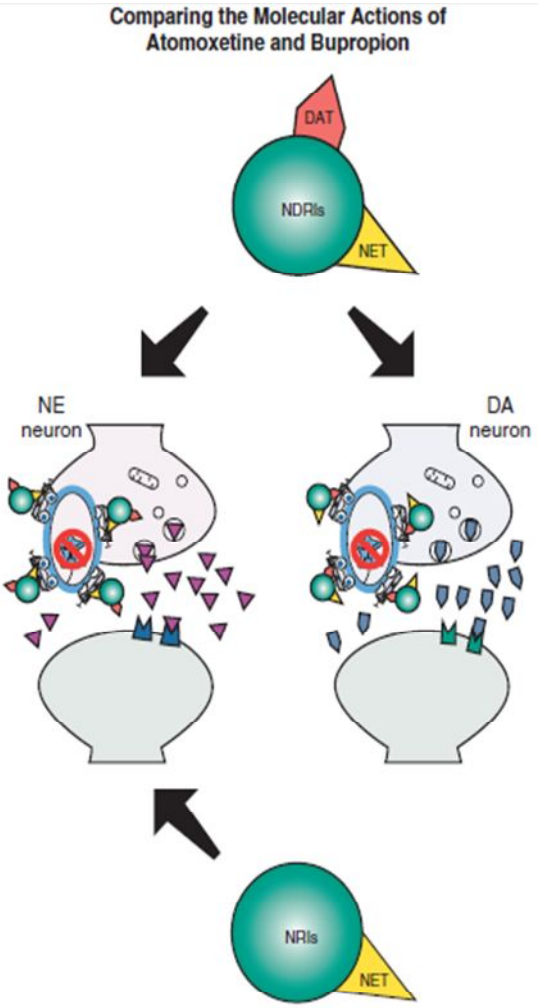


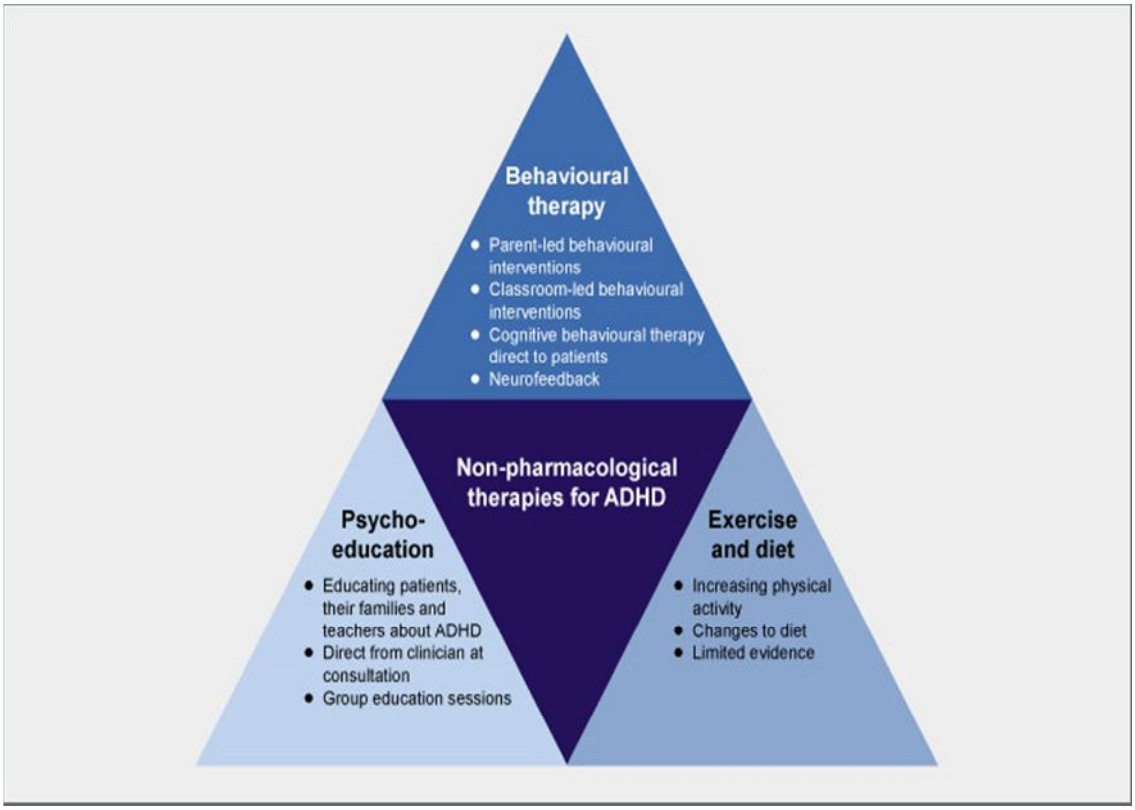
Figure 12-34. Comparing the molecular actions of atomoxetine and bupropion. Atomoxetine is a selective norepinephrine reuptake inhibitor or NRI, while bupropion is a norepinephrine–dopamine reuptake inhibitor or NDRI. Both agents have some pharmacological properties in common, and both of these drugs can have therapeutic effects in the treatment of ADHD.

2.11.2 NON-PHARMACOLOGICAL TREATMENTS FOR ADHD:[28]

Non-pharmacological treatments for ADHD may involve behavioral, psychological, social, educational and lifestyle interventions.

Non-pharmacological treatments for ADHD:

Fig no: 21

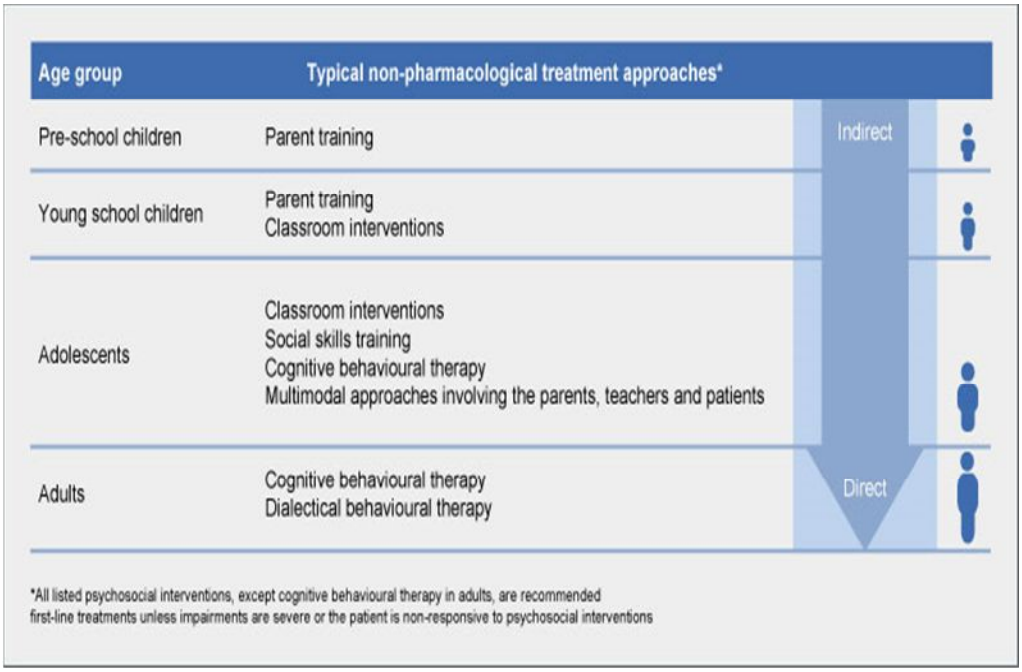


A LIFESPAN APPROACH:

Non-pharmacological interventions should become increasingly focused on the individual as patients mature and become ‘agents of implementation’ in their care.

Non-pharmacological treatments: a lifespan approach. Reproduced with kind permission:

Fig no: 22



PARENT-LED BEHAVIOURAL THERAPY:

Parent-led behavioral therapy aims to treat the core symptoms of ADHD and associated oppositional and non-compliant behaviour. It combines behavior management techniques with novel therapeutic elements based on developmental models of social and cognitive development.

CLASSROOM-BASED BEHAVIOURAL THERAPY:

Classroom-based behavioural therapies are delivered in a real-world situation that provides training on the expected behaviour, within the context in which it is required. These interventions can be delivered by mainstream teachers following appropriate training, and combine behaviour modification and cognitive behavioural modification techniques.

COGNITIVE BEHAVIOURAL THERAPY (CBT):

CBT consists of self-instructional training administered on an individual or group basis. It helps the patient to:

- Develop a more planned and reflective approach to thinking and behaving, including social interactions.

- Adopt a more reflective, systematic and goal-oriented approach to everyday tasks, activities and problem solving, including academic functioning.

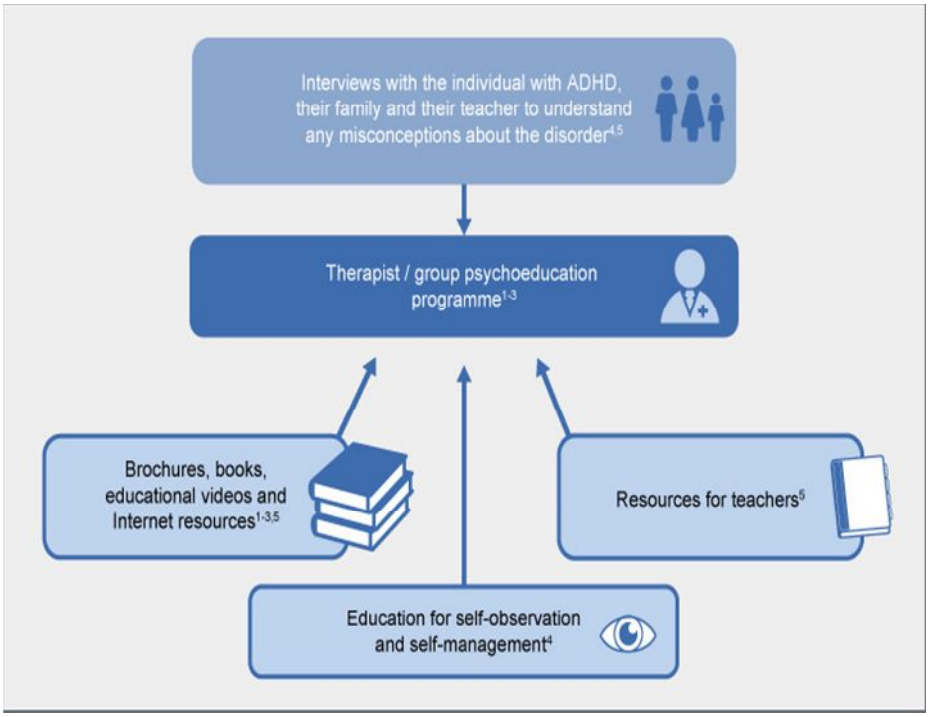
  Regular sessions of CBT have been shown to reduce ADHD symptoms in children, adolescents and adults.

PSYCHOEDUCATION:

Psychoeducation can be viewed as the provision of information regarding ADHD to individuals with the disorder and their families/people close to them. Often seen as an important early step in the treatment of patients with ADHD, psychoeducation may help improve treatment adherence, promote satisfaction with treatment and increase positive functioning outcomes.

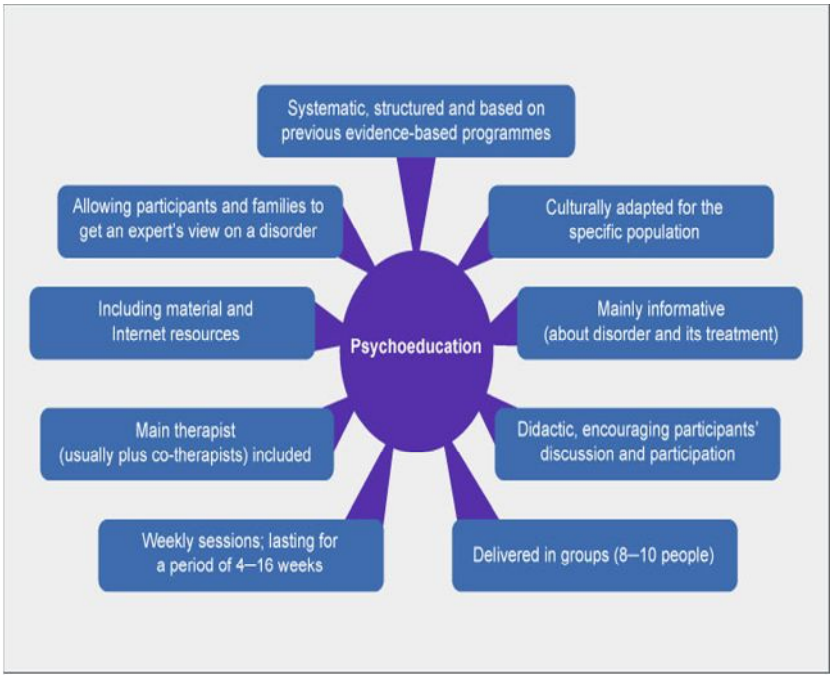
PSYCHOEDUCATION – OVERVIEW:

Fig no: 23



TYPES OF PSYCHOEDUCATION:

Fig no: 24



NEUROFEEDBACK:

Neurofeedback typically involves computer-based exercises, which provide feedback regarding attention levels to enable behavioural training.

Patients' brain activities are measured by electroencephalogram whilst performing a task, usually a computer game, in which patients receive points when their brain activity shows positive changes.

This gives immediate feedback to patients on their level of attention during a task. Patients are then trained to monitor and change their brainwave patterns.

EXERCISE AND DIET:

There is some evidence that physical exercise and dietary interventions may be beneficial for individuals with ADHD, however more research is needed in this area.

2.11.3 MEDICINAL PLANTS TO TREAT ADHD: [29]

1. Acorus calamus
2. Allium sativum
3. Benincasa hispida
4. Bacopa monnieri
5. Caffeine

6. Celastrus paniculatus

7. Chamomile

8. Convolvulus pluricaulis

9. Gingko biloba

10. Ginseng (Siberian)

11. Glycyrrhiza glabra

12. Gotu kola

13. Green oats

14. Hawthrone

15. Herbal tea

16. Kava kava

17. Lavandula stoechas

18. Lemon balm

19. Liquorice

20. Ningdong

21. Nordostachys jatamansi

22. Passion flower

23. Pine bark

24. Red jujulle

25. Rhodiola

26. Salix caprea

27. Scutellaria baicalensis

28. Sideritis scardica

29. Skull cap

30. Tiaoshan liquor

31. Valerian

32. Vitex negundo

2.11.3.1 GINSENG

The irony with Ginseng is that researchers have found it has ability to increase energy and focus while still producing relaxing effects. This makes it one of the better herbal alternatives in improving cognitive performance and attention span among ADHD patients. Evidence shows the ginsenosides it contains stimulate the dopamine pathways that are often deficient in ADHD. Likewise, ginseng has been shown to normalize norepinephrine level which is another neurotransmitter that goes out of balance during ADHD. Consequently, this is why ADHD patients have exhibited improvements in hyperactivity, inattention and immaturity when given Panax Ginseng as alternative treatment.

2.11.3.2 ST. JOHN'S WORT

Like Ginseng, scientists are finding evidence that shows St. John's Wort as a promising herbal treatment for ADHD in terms of improving ADHD symptoms. This plant is considered one the calming herbs to help with sleep difficulties especially among children who are medicated during the day. Aside from also influencing norepinephrine and dopamine levels, research suggests St. John's Wort likewise regulates serotonin and GABA which are the brain's neurotransmitters responsible for improved moods and relaxation respectively.

2.11.3.3 VALERIAN

There is scientific basis that shows valerian, together with passion flower and lemon balm, are useful herbal ADHD aids that promote beneficial physiological actions. Which is why a combination of these mild sedative plants often make up herbal formulas for ADHD to decrease anxiety, nervousness and restlessness.

2.11.3.4 PASSION FLOWER

As with Valerian, Passionflower is an important herbal medicine used for treating generalized anxiety disorder among ADHD sufferers. Studies also demonstrate how passionflower can have pharmacological value in modulating GABA receptors.

2.11.3.5 GINKO BILOBA

Although the potential of Ginko Biloba to aid ADHD is relatively new, studies nonetheless show promising results in significantly improving overall hyperactivity and inattentiveness present in ADHD, with minimal adverse side effects. Scientists believe Ginko Biloba increases blood flow to the nervous system as well as decreases oxidative stress. Moreover, ginko biloba has been shown to boost brain glucose metabolism, have positive

effects on amine neurotransmitters and mood. For optimal results in alleviating symptoms of ADHD, one recent study suggests combining Ginko with American ginseng.

2.11.3.6 LAVENDER

Though mainly used in aromatherapy, lavender still offers promising results in alleviating the suffering of people diagnosed ADHD. To people with ADHD, nervousness and sleeping difficulties are very common. Lavender actually works by relieving tension and inducing sleep. This makes lavender very handy in dealing with such discomforts. Commonly used as oil, lavender can also be used as tea, bath products, scented candles or dried lavenders can just be placed strategically around the house.

Results of the study conducted at the University of Maryland Medical Center revealed that lavender oil has better effects than rosemary oil in mood and cognition. Apart from its calming and relaxing effects, lavender also helped in improving concentration, stabilizing the mood and in reducing anxiety.

2.11.3.7 KAVA KAVA

Known for its calming and relaxing effects, kava kava is a marvelous herb that can be of great benefits to people suffering from ADHD. Kava kava is considered as safe to use even by children. Known to treat anxiety, this herb is very effective in calming patients with ADHD. It can be taken as tea, or in capsule form.

Kava kava is also known for its fast-acting sedation effects and unique relaxing properties. Compared to pharmaceutical ADHD treatments, this herb doesn't cause the sensations to dull, and doesn't impair mental processes. The herb's sedating properties is actually caused by its 6 kavalactone compounds that bind the receptors of the amygdala. This area of the brain regulates fear and anxiety.

Furthermore, kavalactones positively affect the limbic area of the brain that controls hunger, heart rate, sex drive, sleep cycle and other homeostatic mechanisms. When left uncontrolled, these mechanisms can lead to stress, depression and anxiety. Kava kava also works by enhancing one's feelings of well-being and contentment. It also relieves insomnia, tension and nervousness.

2.11.3.8 LICORICE

Licorice is known for its medicinal and healing properties. It is highly recommended for people suffering from hyperactivity, which is a very common symptom of ADHD. It plays

a major role in the proper functioning of the adrenal glands, sweat glands and the endocrine system which facilitates in the occurrence of mood swings.

Licorice also plays a vital role in stabilizing the level of blood sugar. Hyperactivity is usually associated with high level of glucose in the blood.

2.11.3.9 CHAMOMILE

Occasional use of chamomile can also be beneficial in dealing with ADHD. As you may have already known, sleep difficulties are very common to people diagnosed with ADHD who are currently under treatment. When used occasionally or judiciously, chamomile offers a calming effect that enable patients to drowse.

Several scientific studies have supported the claim that intake of chamomile tea can significantly help in relieving the symptoms of ADHD especially in children. Results showed that chamomile makes an excellent treatment for insomnia and anxiety. Furthermore, its soothing properties improve mood states and anxiety levels.

Chamomile actually works on the central nervous system by promoting calmness and easing anxiety. In a study conducted in Italy, the researcher found out that occurrence of certain behaviors like distraction and hyperactivity were significantly reduced when subjects were treated with chamomile.

2.11.3.10 LEMON BALM

This is probably one of the most popular herbs for ADHD in children. It is highly reputed for its positive impact on children suffering from ADHD. Research shows that it helps in improving mental clarity, focus and attention as well as in enhancing one's mental performance. When combined with other mild herbal sedatives like passion fruit and valerian, excellent results are achieved. They are actually beneficial in reducing anxiety, restlessness, fidgeting and nervousness especially in children.

In ancient Europe, this herb is used to improve appetite and sleep as well as in reducing stress and anxiety. Lemon is noted for its ability to improve one's sleep quality without causing daytime sleepiness. This herb is considered safe to use even by children.

2.11.3.11 PINE BARK (PYCNOGENOL)

Pycnogenol is a plant extract from the bark of the French maritime pine tree. Researchers gave 61 children with ADHD either 1 mg of pycnogenol or a placebo once a day

for four weeks in a 2006 study. Results showed that the pycnogenol reduced hyperactivity and improved attention and concentration. The placebo showed no benefits.

Another study found that the extract helped normalize antioxidant levels in children with ADHD. One study published in 2007 showed that pycnogenol lowered stress hormones by 26 percent. It also decreased the amount of the neurostimulant dopamine by nearly 11 percent in people with ADHD.

2.11.3.12BRAHMI (BACOPAMONNIERA)

This Ayurvedic herb has a long history of use as a cognitive enhancer. Research shows that it the protects brain from free radical damage even better than the cognitive-enhancing drug deprenyl (Battacharya), while stimulating improved learning and cognitive function (Kidd).

2.11.3.13GOTU KOLA *(CENTELLA ASIATICA, ALSO HYDROCOTYLE ASIATICA)*

This herb should not to be confused with the caffeine-containing Kola nut. Their triterpenoid glycosides - asiaticoside, madecassoside, and brahmoside - reduce adrenal corticosterone blood levels during stress. They have also been found to be useful for cognitive and nervous disorders and vascular problems of the brain.

2.11.3.14GREENOATS *(AVENASATIVA)*

The fresh green seeds have been used as a mild antispasmotic and nourishing nerve tonic. Its tonic effects are not immediatly stimulating as with caffeine, but are cummulative and resotorative over time with continued use.

2.11.3.15 HAWTHORNE FOR ADHD:

Extracts of the Hawthorne are commonly used in modern herbal medicines. It strengthens the heart and circulatory system. It has a calming effect on the mind. Hence, it is believed that using Hawthorne can help alleviate hyperactivity symptoms and reduce inattentiveness.

2.11.3.16 COMBINATIONS:

Combinations May Work Better:

Some studies have indicated that combining some of these herbs may produce better results than using one alone. A small study in Canada studied children with ADHD who took both American ginseng and Ginkgo biloba twice a day for four weeks. The participants experienced improvements in social problems, hyperactivity, and impulsivity. There are not

many completed studies of the efficacy of herbal ADHD remedies. A 2011 review of complementary treatments for ADHD found that pine bark and a Chinese herbal blend may be effective and brahmi shows promise, but requires further research. With so many options, your best bet may be to check with your doctor, an herbal specialist, or naturopath for more information.

SUMMARY:

Clinical trials and research on herbal aids for ADHD are by no means perfect. But as interest and understanding about ADHD continue to grow, so will scientific knowledge about these herbal treatments be of significant importance as complementary, if not alternative solutions, for ADHD.

3. ANIMAL MODELS OF ADHD: [30]

1. SHR

Neurotransmitter involved:

1. Dopamine
2. Norepinephrine
3. Serotonin
4. Glutamate

2. OTHER ANIMAL MODELS

1. Coloboma mutant mouse
2. 6-OHDA-Lesioned rat
3. DAT-Knockout mouse
4. Poor 5-CSRT task performer
5. Anoxia in neonatal rat

3.1 Introduction:

Although non-human primate brains are closer to human brains than rodents, rodent models of ADHD have the advantage that they are genetically more homogeneous, they are less expensive to maintain, greater numbers of experimental animals are available so they are not used for multiple studies, and much more is known about their neurobiology than primates. The researcher also has better control over variables such as diet, environment, and learning history. Rodent models have simpler nervous systems, they cannot be used to study complex cognitive behaviour like language but their basic behavioural mechanisms are similar to humans.

A list of criteria for an optimal animal model of ADHD was recently suggested

(i) The model should mimic the fundamental behavioural characteristics of ADHD (face validity), impulsiveness should be absent initially and develop gradually over time, sustained attention-deficit should be demonstrated only when stimuli are widely spaced in time, hyperactivity should not be observed in a novel, non-threatening environment, it should develop over time;

(ii) The model should conform to a theoretical rationale for ADHD (construct validity): the two main behavioural processes that are proposed to be major contributory factors

in the aetiology of ADHD, altered rein-forcement of novel behaviour and deficient extinction of previously reinforced behaviour, should be demon- strated;

(iii) The model should predict novel aspects of ADHD behaviour, genetics, and neurobiology (predictive validity)

(iv) It should be neurodevelopmental, preferably a genetic model.

We use the concept 'reinforcer' strictly in a behavioural sense, without making any references to subjective or cognitive states. The alternative concept of 'reward' is more cognitive and may connote several subjective states like pleasure as well as incentive and reinforcer. Therefore, there is not a perfect overlap between reinforcer and reward. We prefer the more descriptive and less ambiguously defined concept of reinforcer rather than reward.

Spontaneously hypertensive rats (SHR) were found to be the best characterized and also currently the most appropriate model of ADHD. SHR fulfill most of the validation criteria listed above and compare well with clinical cases of ADHD. Poor performers in the 5-choice serial reaction time (5-CSRT) task were suggested to be a useful model for the inattentive subtype of ADHD. Other animal models were suggested to provide useful information concerning aspects of ADHD behaviour.

3.2 SHR

SHR exhibit all the behavioural characteristics of ADHD: impaired sustained attention without obvious sensory problems, motor impulsiveness, and hyperactivity that is not present in novel, non-threatening situations but develops over time when reinforcers are infrequent. Similar to children with ADHD, SHR display increased behavioural variability, deficient response re- engagement, and make significantly more errors than controls.

SHR appear to have higher extracellular tonic dopamine in the nucleus accumbens shell. However, consistent with increased DAT1 expression in adult SHR striatum, extracellular dopamine levels are decreased in the caudate nucleus and d-amphetamine-stimulated release of dopamine via DAT1 is greater in SHR striatum than WKY. Evidence suggests that DAT1 is hypo- functional in SHR, since despite the increased number of DATs, inhibition of dopamine uptake by low concentra- tions of methylphenidate or nomifensine increased the electrically-stimulated release of dopamine to the same extent in SHR and WKY nucleus accumbens and caudate- putamen. These findings suggest that increased expression of the DAT1 gene may reflect an attempt to compensate for increased tonic extracellular

dopamine in the nucleus accumbens shell of SHR or increased DAT1 expression may occur in an attempt to compensate for decreased function of DAT1 in adult SHR striatum.

Results obtained with SHR have predicted novel alternatives to existing theories concerning the aetiology of ADHD. A major second messenger system involving calcium signalling is dysfunctional in SHR suggesting that several neurotransmitter systems could be impaired in ADHD. Compared to WKY, SHR have lower brain Ca2+ ATPase activity. Because neurotransmitter release is dependent on calcium influx, a disturbance in the concentration gradient of calcium across the cell membrane may decrease the influx of calcium ions into the cell and impair neurotransmitter release. Decreased calcium influx through NMDA channels would also impair intra- and intercellular signalling as well as LTP, the neuronal analogue of learning.

3.2.1 DOPAMINE

Dopamine neurons play an important modulatory role in the brain. Neurons that release dopamine influence behaviour by exerting modulatory effects on the transfer of information through neuronal circuits that connect functionally distinct cortical areas to specific regions of the striatum in parallel cortico-striato-thalamo-cortical pathways. Dopamine assists in reprogramming the brain by selectively reinforcing the weights of the synapses that are active around the time of behavioural reinforcement.

There are three major dopaminergic systems in the brain, the mesolimbic, mesocortical and nigrostriatal pathways. Mesolimbic dopamine neurons project from the ventral tegmental area of the midbrain (VTA) to limbic areas of the brain. The firing rate of dopamine neurons is increased in response to unexpected reward and decreased when a fully predicted reward is omitted. It has been suggested that deficient reinforcement of appropriate behaviour and/or deficient extinction of previously reinforced behaviour can give rise to ADHD symptoms of delay aversion, hyperactivity in a familiar environment, impulsiveness, deficient sustained attention, increased behavioural variability and failure to extinguish previously acquired behaviour.

3.2.2 Norepinephrine

In addition to the hypothesis that dopaminergic systems are hypofunctional in ADHD, noradrenergic neurons have been suggested to be poorly regulated and hyper-functional in the prefrontal cortex of children with ADHD. Noradrenergic neurons appear to enhance the

signal-to-noise ratio in prefrontal and parietal cortices, amplify responses to attended stimuli, and reduce responses to irrelevant stimuli. Both of these functions are defective in ADHD.

The locus coeruleus diffusely innervates diverse regions throughout the central nervous system including the entire cerebral cortex, various subcortical areas, cerebellum and spinal cord, and plays an important role in attention, arousal, orienting, and vigilance.

The highly specific antagonist of NET1, atomoxetine, is as effective as methylphenidate in treating ADHD, further emphasizing an important role for the noradrenergic system in the disorder. However, atomoxetine also increases synaptic availability of dopamine in the prefrontal cortex which may contribute to its beneficial effects. Drugs used to treat ADHD symptoms are likely to have different effects on different neurotransmitter systems. Drugs that act on the noradrenergic system, such as atomoxetine, tricyclic antidepressants like the NET1 blocker, desipramine, and α_2-adrenoceptor agonists such as clonidine and guanfacine, have a different therapeutic time-course compared to psychostimulants. Methylphenidate produces amelioration of ADHD symptoms within 30 minutes and is short-acting whereas noradrenergic drugs have to be administered for longer periods of time before a therapeutic effect is observed, and improvement is sustained for several months.

3.2.3 SEROTONIN

Brain serotonin (5-hydroxytryptamine, 5-HT) function has been suggested to be altered in SHR. Higher serum testosterone, and lower amygdala serotonin con- tent has been associated with a mutation in the non-pseu-doautosomal region unique to the Y-chromosome of SHR. Administration of a serotonin transporter inhibitor, fenfluramine, evoked less prolactin secretion in SHR than WKY. Acute administration of the selective serotonin reuptake inhibitor, citalopram, reduced hyperactivity of SHR in an elevated plus-maze. However, there was no difference between SHR and WKY in mid-brain, hippocampal, or striatal serotonin concentration or serotonin uptake kinetics. In addition, stressors released serotonin in the locus coeruleus of SHR and WKY rats to the same extent and 5-HT2C receptor function was reported to be unaltered in SHR compared to WKY. These findings do not support a role for serotonin in the aetiology of ADHD symptoms in SHR.

3.2.4 GLUTAMATE

In addition to decreased autoreceptor-mediated inhibition of norepinephrine release from SHR prefrontal cortex slices, glutamate activation of AMPA receptors caused greater

release of norepinephrine from SHR prefrontal cortex slices than WKY. Glutamate is present in micromolar concentrations in the extracellular space out- side the synaptic cleft and regulates tonic dopamine con- centration in the extracellular fluid. Dopamine release is increased by activation of AMPA receptors in rat striatum. Glutamate activation of NMDA receptors upregulates DRD1 function by a direct protein-protein interaction at the carboxy terminals of both receptors. As suggested by Seeman and Madras, the common defect in ADHD could be decreased extracellular dopamine levels. This deficiency could result from increased expression of DAT1, impaired dopamine synthesis, impaired release, or impaired regulation of extra- cellular dopamine by glutamate afferents from the prefrontal cortex, hippocampus, or amygdala.

3.3 PSYCHOSTIMULANTS

Psychostimulants are the most effective drugs used in the treatment of ADHD and provide a powerful means to gain insight into the underlying disturbances of ADHD. D-Amphetamine and methylphenidate reduced the ADHD- like behaviour of SHR [Sagvolden, unpublished; Russell, unpublished]. The increase in DRD1 density observed in SHR striatum is reversed by methylphenidate treatment suggesting that psychostimulants reduce ADHD-like behaviour of SHR by increasing dopamine activation of DRD1 thereby enabling dopamine-mediated LTP and reinforcement mechanisms to take place.

3.4 OTHER ANIMAL MODELS OF ADHD

Several other animal models of ADHD have been pro- posed. These models were developed through genetic manipulation, exposure to toxins, rearing in social isolation, or interference with neurochemical systems. However, several do not satisfy the criteria for animal models of ADHD and have therefore been excluded from the present review. These include the Naples high-excitability rat (NHE), WKHA rat, acallosal mouse, hyposexual rat, PCB-exposed rat, lead-exposed mouse, and rat reared in social isolation.

The reasons for exclusion are briefly as follows: NHE are hyperreactive in a novel environment, they are not hyper-active or impulsive in a familiar environment, and they have not been shown to be impaired in sustained attention. WKHA rats are hyperactive but they are not impulsive. The acallosal mouse becomes hyperactive over time and shows impaired acquisition of conditioned learning tasks.

3.4.1 COLOBOMA MUTANT MOUSE

The SNAP-25 deficient mouse mutant coloboma (Cm/+) is of interest to ADHD because SNAP-25 polymorphisms have been associated with the disorder. SNAP-25 regulates membrane trafficking and is involved in the release of all neurotransmitters as well as regulating trans- location of proteins (e.g. NMDA receptor subunits) to the cell membrane. Altered expression of SNAP-25 will there- fore have diffuse effects on neuronal function. The SNAP- 25 deficient mouse mutant coloboma displays spontaneous hyperactivity but lacks impulsiveness and has not been shown to have problems with sustained attention. Although the SNAP-25 deficient mouse does not model ADHD symptoms specifically, it may nevertheless serve as a useful model of non-specific brain dysfunction such as minimal brain disorder (MBD).

3.4.2 6-OHDA-LESIONED RAT

Neonatal 6-OHDA-lesioned rats are not impulsive but they display hyperactivity and impaired learning in a spatial discrimination task, which improves after methylphenidate or d-amphetamine treatment. Rat pups lesioned on postnatal day 1 displayed hyperactivity in adulthood. They showed an initial decrease in spontaneous motor behaviour when placed in a novel environment, but after repeated testing their activity was increased relative to controls. Hyperactivity was accompanied by decreased dopamine, increased DRD4, and increased serotonin transporter binding in striatum but not cerebral cortex. Hyperactivity was not altered

by DAT1 inhibitors but was greatly reduced by DRD4 antagonists as well as inhibitors of SERT1 and NET1. These findings suggest that psychostimulants reduce hyperactivityb of 6-OHDA lesioned rats not by inhibiting DAT1 but by inhibiting norepinephrine and serotonin transporters. Inhibition of NET1 would reduce dopamine uptake into noradrenergic terminals in several brain areas including prefrontal cortex and nucleus accumbens.

3.4.3 DAT-KNOCKOUT MOUSE

DAT-knockout (DAT-KO) mice lack the gene that encodes DAT. These mice have been suggested as a model for ADHD because they are hyperactive in novel situations, have an impaired extinction of responses in operant food reinforcement tasks. They are also impaired in learning and memory tasks. Impulsiveness has not been systematically investigated in DAT- KO mice. Although the absence of DAT is an extreme model of reduced midbrain DAT binding in adolescents with ADHD it also contrasts with several studies that found increased DAT in striatum of ADHD children and adults. The DAT-KO mouse nevertheless pro- vides useful information concerning the neurobiological consequences of impaired DAT function.

3.4.4 POOR 5-CSRT TASK PERFORMER

Rats that are selected for poor performance when trained in the 5-CSRT task provide a useful model of ADHD in that they are selected for deficient sustained attention, they show poor choice accuracy towards the end of testing sessions, and they demonstrate impulsiveness (premature responding). Methylphenidate treatment improved accuracy and reduced impulsiveness (at low doses) in poor performers. Poor 5-CSRT task per- formers are not hyperactive and therefore may serve as a model of the inattentive subtype of ADHD.

Evidence suggests that dopamine function is reduced in poor performers of the 5-CRST task and that 5-HT2A antagonists may be beneficial in the treatment of ADHD.

3.4.5 ANOXIA IN NEONATAL RAT

Anoxia increases the risk of ADHD. Neonatal anoxia caused a sequence of acute and persistent neurochemical changes in rat monoaminergic systems as well as transient hyperactivity and spatial memory impairment that persisted into adulthood. Acute anoxia caused a transient decrease followed by an increase after 1 week in cerebellar norepinephrine levels. At the same time, serotonin levels decreased while its metabolite, 5-hydroxy-indoleacetic acid (5-HIAA), increased. Striatal dopamine and metabolite concentrations

decreased and then dopamine metabolites increased post ischaemia. The increase in serotonin and dopamine metabolites persisted into adulthood, suggesting that dopamine turnover is increased. Tyrosine hydroxylase mRNA levels were increased in VTA and substantia nigra of perinatally asphyxiated rats suggesting increased dopamine synthesis consistent with increased turnover. However, DRD1 and DRD2 mRNA levels were increased in the striatum suggesting impaired release of dopamine. These findings demonstrate the complex temporal sequence of compensatory changes that occur in monoaminergic systems following perinatal insult to the nervous system and implicate all three monoaminergic systems in spatial memory impairment.

3.5 INSIGHT PROVIDED BY ANIMAL MODELS OF ADHD:

One of the most important findings is the fact that animal models of both inattentive and hyperactive/impulsive subtypes of ADHD respond differently to psychomotor drugs when compared to controls suggesting that they have altered neurotransmitter systems in the brain. This emphasizes the need to study animal models of ADHD rather than normal animals in order to gain insight into the mechanisms that underlie the beneficial effects of drugs used to treat children with ADHD.

4. LITERATURE REVIEW FOR ADHD:

1. **Enhanced learning and memory of normal young rats by repeated oral administrationof KrillPhosphatidylserine.**

Park, H *et al* reported activity by Using Krill Phosphatidylserine improved cognitive function in rats, better retention, learning, and memory.

Nutritional Neuroscience, 16(2), 47-53.

2. **Influence of phosphatidylserine on cognitive performance and cortical activity after inducedstress.**

Baumeister *et al* reported that after using phosphatidylserine for 42 days, Beta-1 activity was significantly decreased in brain before and after stress, which suggests relaxation was increased in stressful situations.Support for improved cognitive function was also found.

Nutritional Neuroscience, 11(3), 103-110. (2008).

3. **A Systematic Review of Magnesium Therapy for Treating Attention Deficit Hyperactivity Disorder.**

Ghanizadeh, A*et al* reported that magnesium supplements have potential to help those with ADHD, as many children in these studies suffered from magnesium deficiency.

Of Archives Iranian Medicine (AIM), 16(7), 412-417. (2013).

4. **Effects of Zinc and Ferritin Levels on Parent and Teacher Reported Symptom Scores in Attention Deficit Hyperactivity Disorder.**

Oner, O *et al* reported that low zinc levels related to higher hyperactivity, as well as anxiety.

Child Psychiatry & Human Development, 41(4), 441-447. doi:10.1007/s10578-010-0178-1 (2010).

5. **Role of zinc in the pathogenesis of attention-deficit hyperactivity disorder: Implications for research and treatment.**

Lepping, P *et al*discussed that zinc receptors in the brain linked to hyperactivity and attention and how zinc supplements appeared to improve these symptoms in patients.

CNS Drugs, 24(9), 721- 728. (2010).

6. **Nutritional and dietary influences on attention deficit hyperactivity disorder.**

Sinn, N. *et al*reported that support for nutritional deficiencies causing symptoms of ADHD, with Zinc and Omega 3 fatty acids deficiencies especially causing problems.

Nutrition Reviews, 66(10), 558-568. (2008).

7. **Omega-3 fatty acids in ADHD and related neurodevelopmental disorders.**

Richardson, A. J. *et al*reported that some support for omega-3s alleviating ADHD symptoms, at least useful as supplementary to other treatment, although more research is needed.

International Review Of Psychiatry, 18(2), 155-172. (2006).

8. **Omega-3 fatty acid supplementation for the treatment of children with attention- deficit/hyperactivity disorder symptomatology: systematic review and meta-analysis.**

Reading. R *et al* reported that small but significant improvement of ADHD symptoms, especially when paired with Eicosapentaenoic acid. Enough support for its use that it could be used along with other ADHD treatments or on its own could possibly show some improvement.

Child: Care, Health & Development, 39(1), 150-151. doi:10.1111/cch.12022. (2013).

9. **The spontaneously hypertensive rat model of ADHD--the importance of selecting the appropriate reference strain. Neuropharmacology.**

Sagvolden T *et al*reported that the use of WKY/NCrl, outbred Wistar, Sprague Dawley or other rat strains as controls for SHRs may produce spurious neurobiological differences. Consequently, data may be misinterpreted if insufficient care is taken in the selection of the control group. It appears likely that the use of different control strains may underlie some of the discrepancies in results and interpretations in studies involving the SHR and WKY. Finally, we argue that WKY rats obtained from Charles River, Germany (WKY/NCrl) provide a promising model for the predominantly inattentive subtype of ADHD (ADHD-PI); in this case also the WKY/NHsd substrain should be used as control.

2009;57(7-8):619-26. doi: 10.1016/j.neuropharm.2009.08.004. Epub 2009 Aug19.

10. **The control of responsiveness in ADHD by catecholamines: evidence for dopaminergic, noradrenergic and interactive roles.**

Oades RD *et al* reported that the neurobiological bases of attention deficit hyperactivity disorder (ADHD) from the viewpoint of the neurochemistry and psychopharmacology of the catecholamine-based behavioural systems. The contributions of dopamine (DA) and noradrenaline (NA) neurotransmission to the motor and cognitive symptoms of ADHD (e.g. hyperactivity, variable and impulsive responses) are studied in rodent and primate models.

These models represent elements of the behavioural units observed in subjects with ADHD clinically, or in laboratory settings (e.g. locomotion, changed sensitivity/responsivity to novelty/reinforcement and measures of executive processing). In particular, the models selected emphasize traits that are strongly influenced by mesocorticolimbic DA in the spontaneously hypertensive (SHR) and the Naples high excitability (NHE) rat lines. In this context, the mode of action of methylphenidate treatment is discussed. We also describe current views on the altered control by mesolimbic catecholamines of appropriate and inappropriate goal-directed behaviour, and the tolerance or intolerance of delayed reinforcement in ADHD children and animal models. Recent insights into the previously underestimated role of the NA system in the control of mesocortical DA function, and the frontal role in processing information are elaborated.

Dev Sci. 2005 Mar;8(2):122-31.

11. **Acoustic noise improves motor learning in spontaneously hypertensive rats, a rat model of attention deficit hyperactivity disorder.**

Söderlund GB *et al* reported that the acoustic noise benefit previously reported in children with ADHD is shared by the SH rat model of ADHD, and the effect is in the same range as that of stimulant treatment. Acoustic noise may be useful as a non-pharmacological alternative to stimulant medication in the treatment of ADHD.

Behav Brain Res. 2015 Mar 1;280:84-91. doi: 10.1016/j.bbr.2014.11.032. Epub 2014 Nov 29.

12. **Early life stress induces attention-deficit hyperactivity disorder (ADHD)-like behavioral and brain metabolic dysfunctions: functional imaging of methylphenidate treatment in a novel rodent model**

J. Bock *et al* reported that the elevating dopamine in ELS animals by methylphenidate normalized locomotor hyperactivity and attention-deficit and ameliorated brain metabolic hypoactivity in a dose-dependent manner.

Brain Struct Funct. 2017; 222(2): 765–780.Published online 2016 Jun 16.

13. **Using the Morris water maze to assess spatial learning and memory in weanling mice.**

Barnhart CD *et al* reported that Morris water maze can be used to assess spatial learning and memory in weanling mice, providing a potentially powerful experimental

approach for examining the influence of genes, environmental factors and their interactions on the development of learning and memory.

PLoS One. 2015 Apr 17;10(4):e0124521. doi: 10.1371/journal.pone.0124521. eCollection 2015

14. **Atomoxetine increases histamine release and improves learning deficits in an animal model of attention-deficit hyperactivity disorder: the spontaneously hypertensive rat.**

Liu LL *et al* reported that the current study provides further support for the notion that the therapeutic effect of atomoxetine could involve activation of histamine neurotransmission within the prefrontal cortex.

Basic Clin Pharmacol Toxicol. 2008 Jun;102(6):527-32.

15. **Differences in the performance of NK1R−/− ('knockout') and wildtype mice in the 5-Choice Continuous Performance Test.**

Ashley J. Porter *et al* reported that the mice were tested for the first time, neither false alarms nor premature responses was higher in NK1R−/− mice than wildtypes but, as in the 5-CSRTT, the latter behaviour was strongly dependent on time of day. NK1R−/− mice expressed excessive perseveration during all stages of the 5C-CPT. This behaviour is thought to reflect compulsive checking, which is common in ADHD patients. These findings point to differences in the 5-CSRTT and 5C-CPT protocols that could be important for distinguishing why the cognitive performance and response control of NK1R−/− mice differs from their wildtypes. The results further lead to the prediction that ADHD patients with polymorphism of the TACR1 gene (the human equivalent of Nk1r) would express more perseveration, but not false alarms, in Continuous Performance Tests when compared with other groups of subjects.

Behav Brain Res. 2016 Feb 1; 298(Pt B): 268–277.

16. **Effects of neonatal treatment with 6-hydroxydopamine and endocrine disruptors on motor activity and gene expression in rats.**

Masuo Y *et al* reported that Rats received an endocrine disruptor (87 nmol), such as bisphenol A, nonylphenol, p-octylphenol, or diethylhexylphthalate, which also caused motor hyperactivity at 4 weeks. The effects of bisphenol A on motor activity were dose-dependent from 0.87 to 87 nmol. The phenols caused a deficit in dopamine neurons, similarly to the

deficit caused by 6-hydroxydopamine. Gene-expression profiles after treatment with endocrine disruptors showed variation and differed from those of 6-hydroxydopamine. The results suggest that neonatal treatment with environmental chemicals can generate an animal model of attention-deficit hyperactivity disorder, in which clinical symptoms are pervasive.

Neural Plast. 2004;11(1-2):59-76.

17. **Role of dopamine D(4) receptors in motor hyperactivity induced by neonatal 6-hydroxydopamine lesions in rats.**

Zhang K *et al*reported that The role of dopamine D(4) receptors in behavioral hyperactivity was investigated by assessing D(4) receptor expression in brain regions and behavioral effects of D(4) receptor-selective ligands in juvenile rats with neonatal 6-hydroxydopamine lesions, a laboratory model for attention deficit-hyperactivity disorder (ADHD). Autoradiographic analysis indicated that motor hyperactivity in lesioned rats was closely correlated with increases in D(4) but not D(2) receptor levels in caudate-putamen. D(4)-selective antagonist CP-293,019 dose-dependently reversed lesion-induced hyperactivity, and D(4)-agonist CP-226,269 increased it. These results indicate a physiological role of dopamine D(4) receptors in motor behavior, and may suggest much-needed innovative treatments for ADHD.

Neuropsychopharmacology. 2001 Nov;25(5):624-32.

18. **Effects of norepinephrine and serotonin transporter inhibitors on hyperactivity induced by neonatal 6-hydroxydopamine lesioning in rats.**

Davids E *et al*reported that the Selective DA transport inhibitors GBR-12909 and amfonelic acid greatly stimulated motor activity in sham control subjects, too, but did not antagonize hyperactivity in lesioned rats. In contrast, all selective 5-HT and NE transporter antagonists tested greatly reduced motor hyperactivity in 6-OHDA lesioned rats but did not alter motor activity in sham controls. The findings indicate that behavioral effects of stimulants in young rats with neonatal 6-OHDA lesions may be mediated by release of NE or 5-HT and support interest in using drugs that increase activity of norepinephrine or serotonin to treat ADHD.

J Pharmacol Exp Ther. 2002 Jun;301(3):1097-102.

19. Atomoxetine blocks motor hyperactivity in neonatal 6-hydroxydopamine-lesioned rats: implications for treatment of attention-deficit hyperactivity disorder.

Moran-Gates T *et al*reported that atomoxetine greatly reduced motor hyperactivity in 6-OHDA-lesioned rats while exhibiting transient sedative effects in sham controls. The observed effects in this animal model for ADHD are consistent with the emerging clinical use of atomoxetine as a novel, non-stimulant treatment for ADHD.

Int J Neuropsychopharmacol. 2005 Sep;8(3):439-44. Epub 2005 Apr 7.

20. Spontaneously hypertensive rats do not predict symptoms of attention-deficit hyperactivity disorder.

van den Bergh FS *et al* reported that the validity of the Spontaneously Hypertensive rat (SHR) as a model for Attention Deficit Hyperactivity Disorder (ADHD) is explored by comparing the SHR with Wistar-Kyoto (WKY) and Wistar rats in a number of different tests. In the open field, SHR are hyperactive compared to both Wistar and WKY, but only at specific ages. At those ages, methylphenidate (1mg/kg) did not attenuate hyperactivity. Subsequently, a dose response study of methylphenidate (0.1-10mg/kg) was conducted in the Differential Reinforcement of Low-rate responding (DRL)-72s and five-choice serial reaction time tests (5-CSRTT). Compared to WKY but not Wistar rats, SHR performed worse on the DRL-72s. Performance was not improved by methylphenidate (0.1-1.0mg/kg). In the 5-CSRTT, attentional performance was similar for all rat strains, but Wistar rats made more impulsive responses than both the SHR and the WKY. Methylphenidate only attenuated impulsivity in Wistar rats. Because SHR do not consistently display symptoms of ADHD across the different tests, and methylphenidate effects were observed in both WKY and Wistar rats, but not in SHR, we conclude that SHR is not a representative animal model for ADHD.

Pharmacol Biochem Behav. 2006 Mar;83(3):380-90. Epub 2006 Mar 6.

21. Proposed animal model of attention deficit hyperactivity disorder.

Kostrzewa RM *et al*reported that oral activity dose-effect curves established that 5,7-DHT attenuated DA D1 receptor supersensitivity and further sensitized 5-HT2c receptors. Acute treatment with dextroamphetamine (0.25 mg/kg SC) reduced locomotor time in 6-OHDA + 5,7-DHT-lesioned rats to 76 +/- 37 s ($p < 0.001$). Striatal DA was reduced by 99% and 5-HT

was reduced by 30% (vs. 6-OHDA group). Because combined 6-OHDA (to neonates) and 5,7-DHT (to adults) lesions produce intense hyperlocomotion that is attenuated by amphetamine, we propose this as a new animal model of ADHD. The findings suggest that hyperactivity in ADHD may be due to injury or impairment of both DA and 5-HT neurons.

Brain Res Bull. 1994;34(2):161-7.

22. **Caffeine improves spatial learning deficits in an animal model of attention deficit hyperactivity disorder (ADHD) -- the spontaneously hypertensive rat (SHR).**

Prediger RD *et al*reported that Pre-training administration of caffeine (1-10 mg/kg i.p.) improved this spatial learning deficit in SHR, but did not alter the WIS performance. In contrast, post-training administration of caffeine (3 mg/kg i.p.) did not alter the SHR test performance, but increased memory retention in WIS rats. No dose of caffeine tested altered the mean blood pressure of WIS or SHR. These results demonstrate a selective spatial learning deficit in SHR which can be attenuated by pre-training administration of caffeine. In addition, the present findings indicate that the spatial learning deficit in SHR is not directly related to hypertension.

Int J Neuropsychopharmacol. 2005 Dec;8(4):583-94. Epub 2005 May 9.

23. **Motor activity and gene expression in rats with neonatal 6-hydroxydopamine lesions.**

Masuo Y *et al*reported that in the striatum the neurotransmitters glutamate, GABA and tachykinin may play crucial roles in motor hyperactivity during the juvenile period. Several classes of neurotransmitters, including dopamine and peptides, may be involved in compensatory mechanisms during early adulthood. These data may prompt further neurochemical investigations in hyperkinetic disorders.

J Neurochem. 2004 Oct;91(1):9-19.

24. **Combined effects of marijuana and nicotine on memory performance and hippocampal volume.**

Francesca M.Filbey *et al*reported that the controls showed a trend for larger hippocampal volumes being associated with better memory scores, while MJ + Nic users showed a unique inversion, whereby smaller hippocampal volume was associated with better memory. Overall, results suggest abnormalities in the brain-behavior relationships underlying memory

processes with combined use of marijuana and nicotine use. Further research will need to address these complex interactions between MJ and nicotine.

Behavioural Brain Research Volume 293, 15 October 2015, Pages 46-53

25. **Effect of atomoxetine on hyperactivity in an animal model of attention-deficit/hyperactivity disorder (ADHD).**

Moon SJ *et al*reported that The motor activity improved continuously in the group treated with atomoxetine at a dose of 1 mg/Kg/day than in the groups treated with atomoxetine at a dose of 0.25 mg/Kg/day or 0.5 mg/Kg/day. With respect to DA D2 receptor immunohistochemistry, we observed significantly increased DA D2 receptor expression in the PFC, striatum, and hypothalamus of the SHRs as compared to the WKY rats. Treatment with atomoxetine significantly decreased DA D2 expression in the PFC, striatum, and hypothalamus of the SHRs, in a dose-dependent manner.

PLoS One. 2014 Oct 1;9(10):e108918

26. **Effects of dopamine D4 receptor-selective antagonists on motor hyperactivity in rats with neonatal 6-hydroxydopamine lesions.**

Kehong Zhang *et al*reported that Motor hyperactivity in this ADHD model was selectively antagonized by three of four dopamine D4 receptor antagonists evaluated, encouraging clinical assessment of D4 antagonists in patients with ADHD.

Psychopharmacology April 2002, Volume 161, Issue 1, pp 100–106

27. **Rodent models: utility for candidate gene studies in human attention-deficit hyperactivity disorder (ADHD).**

Mill J *et al*reported that rodents are excellent tools for the identification of novel ADHD candidate genes. While not yet widely used to identify genes for ADHD-like behaviors in rodents, quantitative trait loci (QTL) mapping approaches using recombinant inbred strains, heterogeneous stock mice, and chemically mutated animals have the potential to revolutionize our understanding of the genetic basis of ADHD.

J Neurosci Methods. 2007 Nov 30;166(2):294-305. Epub 2007 Jan 17.

28. **Strengths and limitations of genetic models of ADHD.**

Raul R. Gainetdinov *et al*reviewed that mutants as well as other mouse models of DAT dysfunction provided an opportunity to investigate the neuronal circuitry and molecular

mechanisms involved in the inhibitory action of psychostimulants on hyperactivity. Several additional knockout and transgenic mouse models have been proposed to model ADHD. Strengths and limitations of currently available genetic mouse models of ADHD are discussed.

ADHD Attention Deficit and Hyperactivity Disorders March 2010, Volume 2, Issue 1, pp 21–30

29. Effects of Red Ginseng on Neonatal Hypoxia-induced Hyperacitivity Phenotype in Rats.

Kim, Hee-Jin *et al*reported that in neonatal hypoxia-induced rats, expression of the norepinephrine transporter in the forebrain was increased, and red ginseng treatment partially prevented its up-regulation, while increasing its level in the control rats. Taken together, these results suggest that red ginseng extract decreased the neonatal hypoxia-induced hyperactivity phenotype, although it increased locomotor activity in normal animals.

Journal title : Journal of Ginseng Research Volume 34, Issue 1, 2010, pp.8-16 Publisher : The Korean Society of Ginseng

30. A simple behavioral paradigm to measure impulsive behavior in an animal model of attention deficit hyperactivity disorder (ADHD) of the spontaneously hypertensive rats.

Kim P *et al*reported that the SHR was more impulsive than the WKY as it demonstrated more "drinking attempts" and drinking frequency. Methylphenidate, the most widely used ADHD medication, significantly reduced drinking frequency of both SHR and WKY in the EFSDT. Thus, the present assay may be considered as another behavioral tool to measure impulsivity in animal disease models, especially in the context of ADHD.

Biomol Ther (Seoul). 2012 Jan;20(1):125-31.

31. Lobeline Effects on Cognitive Performance in Adult ADHD.

Catherine A. Martin *et al*reported that In preclinical studies, lobeline inhibited hyperactivity induced by nicotine and amphetamine, and improved performance and learning in studies utilizing radial-arm maze and spatial-discrimination water maze. This laboratory proof-of-concept study investigated lobeline as a treatment for ADHD symptoms in adults (31.11 ± 7.08 years).

J Atten Disord. : 10.1177/1087054713497791. Published online 2013 August 21.

5. (6-OHDA HBr Lesioned Neonates as an Experimental Animal Model):

5.1 MECHANISM OF ACTION OF 6-OHDA: [31]

Cell death induced by 6-hydroxydopamine (6-OHDA) is thought to be caused by reactive oxygen species (ROS) derived from 6-OHDA autooxidation and by a possible direct effect of 6 OHDA on the mitochondrial respiratory chain. However, the process has not been totally clarified. In rat primary mesencephalic cultures, we observed a significant increase in dopaminergic (DA) cell loss 24 h after administration of 6-OHDA (40 lmol/L) and a significant increase in NADPH subunit expression, microglial activation and superoxide anion/superoxide derived ROS in DA cells that were decreased by the NADPH inhibitor apocynin.

5.2 STANDARD PROCEDURE TO INDUCE HYPERACTIVITY: [32]

Timed pregnant CD albino rats were obtained from Charles River Laboratories (Research Triangle, NC). Animals were housed at 22±lºC, under a 12L: 12D cycle (on at 0700 h) and were provided free access to food and water. At birth, litters were randomized, so that each dam had rats from several litters, with each reconstituted litter consisting of 10 pups. At 3 days after birth, rats were pretreated with Desipramine HCl (20 mg/kg IP, base) 1 h before intracerebroventricular (ICV) 6-OHDA HBr (67 pg in each lateral ventricle, base) in 0.9% saline containing 0.1% ascorbic acid as vehicle.

6. REVIEW AND LITERATURE OF PLANTS:

6.1 REVIEW AND LITERATURE OF ALLIUM SATIVUM:

1. **Allium Sativum reduces toxic effects**

Ncir. M *et al* has investigated the in vitro and the in vivo antioxidant capacities of Allium sativum (garlic) extract against deltamethrin-induced oxidative damage in rat's brain and kidney and reported that the co-administration of garlic extract reduced the toxic effects in brain and kidney tissues induced by deltamethrin.

Arch Physiol Biochem. 2017 Sep 18:1-11

2. **Allium Sativum has neuroprotective effects:**

BC Mathew *et al* has reported that the broad range of anti-atherogenic, antioxidant and anti-apoptotic protection afforded by garlic may be extended to its neuroprotective action, helping to reduce the risk of dementia, including vascular dementia and AD.

Libyan J Med. 2008; 3(1): 23–33.

3. **Allium Sativum has neuroprotective effects:**

Belle LP *et al* has reported that Garlic alcoholic extract may be effective in reducing the effect of methylmercury-induced adenosine deaminase, which may be due to its sulphur-containing compounds.

Basic Clin Pharmacol Toxicol. 2009 May;104(5):408-13.

4. **Allium Sativum has hypoglycemic effects:**

Khaled Al-Qattan *et al* has reported hypoglycaemic effects of garlic and ginger concurred with attenuation in the progression of diabetic structural nephropathy.

e-SPEN, the European e-Journal of Clinical Nutrition and Metabolism Volume 3, Issue 2, April 2008, Pages e62-e71

5. **Allium Sativum has a role in neuropsychopharmacology:**

Jagdeep S. Dua *et al* has reviewed that these plants Allium sativum, Bacopa monnierae, Centella asiatica, Celastrus paniculatus, Nicotiana tabaccum, Withania somnifera, Ricinus communis, Salvia officinalis, Ginkgo biloba, Huperiza serrata, Angelica sinensis, Korean ginseng, Uncaria tomentosa, Hypericum perforatum, Physostigma venosum, Acorus calmus, Curcuma longa, Terminalia chebula, Crocus sativus, Enhydra fluctuans, Vitex negandu, Valeriana wallichii, Glycyrrhiza glabra has potential role inneuropsychopharmacology.

Asian Journal of Pharmaceutical and Clinical Research Volume 2, Issue 2, April- June, 2009

6. **Allium Sativum has depressant effect and toxic in high doses:**

Donatien Gatsing *et al* has reported that aqueous extract of allium sativum may have a depressant effect on the central nervous system, a sedative effect, and may induce a decrease in plasma prostaglandin levels. Also, this extract, at high doses, may induce injury to liver, spleen and lungs, loss of appetite, and anemic conditions.

Cameroon Journal of Experimental Biology Journal Home Vol 1, No 1 (2005)

6.2 REVIEW AND LITERATURE OF BENINCASA HISPIDA:

1. Benincasa hispida has anxiolytic effects:

S K Nimbal *et al* has reported that the extract administered orally was able to increase the percentage of time spent and the percentage of open arm entries in the elevated plus maze, as well as increase the time spent in the illuminated side of the light-dark test.

International Journal of Pharmacy and Pharmaceutical Science Research 2011; 1 (3) 93-97

2. Benincasa hispida has anti-compulsive effects:

SHIKHA GIRDHAR *et al* Benincasa hispida may be useful in the treatment of obsessive-compulsive disorder. Therefore, the influence of methanolic extract of Benincasa hispida fruit was investigated on the marble-burying behavior of mice in a well-accepted model of obsessive-compulsive behavior, due to its high face and predictive validity.

Acta Poloniae Pharmaceutica ñ Drug Research, Vol. 67 No. 4 pp. 417ñ421, 2010

3. Benincasa hispida a review on its pharmacological importance:

Ali Esmail Al-Snafi *et al* has reviewed that the plant exerted many pharmacological activities, including central nervous effects (anxiolytic , muscle relaxant , antidepressant , in the treatment of Alzheimer's disease and to minimize opiates withdrawal signs), antioxidant, anti-inflammatory, analgesic, antiasthmatic, diuretic , nephroprotective , antidiabetic , hypolipidemic and antimicrobial effects . This review was designed to highlight the chemical constituents and pharmacological effects of Benincasa hispida.

Ali Esmail Al-Snafi / International Journal of Pharma Sciences and Research (IJPSR) ISSN : 0975-9492 Vol 4 No 12 Dec 2013

4. **Benincasa hispida has anticonvulsant activity:**

Shivkumar Ladde *et al* has reported that the ethanolic extract of Benincasa Hispida contains pharmacologically active substance(s) like triterpenoids, flavonoids, glycosides and steroids, which may be valuable in the treatment of convulsive disorders, especially Grand mal epilepsy.

Shivkumar Ladde *et al*. IRJP 2011, 2 (12), 166-168

5. **Benincasa hispida is effective in Colchicine Induced Experimental Rat Model of Alzheimer`s Disease:**

Chandan Roy *et al* reported that at a dose of 400 mg kg-1 body weight BH has protective effect on colchicine induced Alzheimer`s disease.

International Journal of Pharmacology Year: 2008 Volume: 4 Issue: 4 Page No.: 237-244

6. **Benincasa hispida has anti-depressant activity:**

Dinesh Dhingra *et al* reported that the methanolic extract of Benincasa hispida showed significant antidepressant-like activity in mice probably by inhibiting MAO-A, and through interaction with dopaminergic, α1-adrenergic, serotoninergic, and GABAergic systems.

J Pharmacol Pharmacother. 2012 Jan-Mar; 3(1): 60–62.

6.3 REVIEW AND LITERATURE OF CELASTRUS PANICULATUS:

1. **Celastrus Paniculatus Reversed impairment in scopolamine-induced deficits:**

Gattu M *et al* reported that the seed oil of Celastrus Paniculatus, when administered chronically, selectively reversed the impairment in spatial memory produced by acute central muscarinic receptor blockade, supporting the possibility that one or more constituents of the oil may offer cognitive enhancing properties. The neural mechanism underlying the reversal of scopolamine's mnemonic effects by CP is not yet known, but it is not related to an anticholinesterase-like action.

Pharmacol Biochem Behav. 1997 Aug;57(4):793-9.

2. **Celastrus Paniculatus has an effect on passive avoidance performance:**

   Nalini K *et al* reported that Celastrus oil causes an overall decrease in the turnover of all the three central monoamines and implicate the involvement of these aminergic systems in the learning and memory process.

J Ethnopharmacol. 1995 Jul 7;47(2):101-8.

3. **Celastrus Paniculatus protects neuronal cells in H2O2 induced toxicity:**

Godkar PB *et al* has reported that CPO (Celastrus paniculatus seed oil), ME (methanolic extract), and EE (ethanolic extract) protected neuronal cells against H(2)O(2)-induced toxicity in part by virtue of their antioxidant properties, and their ability to induce antioxidant enzymes. However, CPO, which exhibited the least antioxidant properties, was the most effective in preventing neuronal cells against H(2)O(2)- and glutamate-induced toxicities. Thus, in addition to free-radical scavenging attributes, the mechanism of CP seed component (CP-C) neuroprotection must be elucidated.

Phytomedicine. 2006 Jan;13(1-2):29-36. Epub 2005 Jun 28.

4. Celastrus Paniculatus has adaptogenic effect in drug induced narcosis in mice:

F. Ahumada *et al* reported that depressing effects of the extracts of Ocimun sanctum (O), Schizandra chinensis (S), Sedum rosea (R), Withania somnifera (W), Aralia elata (A), and Celastrus paniculatus (C) upon the mouse central nervous system (CNS) was evaluated. Recovery time of the righting reflex after the injection of a single dose of pentobarbital (50 mg/kg i.p.) was compared to the effect induced by that drug and the extracts. Decreasing or increasing the time for mouse recovery was evaluated in relation to dose and sex. The results demonstrate a significant ($p \leqslant 0.05$) increase of the narcosis time with all the extracts. The recovery of the righting reflex was sex and dose-dependent. These plant extracts have a clear synergism with pentobarbital in depressing the mouse CNS response.

Phytotherapy research Volume 5, Issue 1 February 1991 Pages 29–31

5. Celastrus Paniculatus is found to be effective in the treatment for ADHD:

Girish Sudhakar Soman *et al* reported that a herbal composition comprising supercritical fluid extracts/oils of Jyotishmati, Bacopa monnieri, Ginger Oil, Flax Seed oil, Rosemary Ext and Vitamin E is effective in the treatment of symptoms associated with Attention Deficit disorder (ADD) and Attention deficit/hyperactivity disorder (ADHD).

Publication number-US20120189723 A1,Application number-US 13/340,188,Publication date-26 Jul 2012.

6. Celastrus Paniculatus is effective in alopecia:

Harpreet Kaur *et al* reported that exploring the herbal drugs for the promotion of hair growth is the vital need of this era. The potential of end number of herbal drugs in hair growth promotion has been studied. But still more scientific documentation of herbal/ayurvedic drugs is needed for the same. This can be attained by careful and accurate characterization of the active phytoconstituents, elucidation of molecular mechanism of their actions, demonstrations of the real efficacy by in vivo studies on proper animal models of hair loss and finally by demonstration of their safety and effectiveness in clinical trials.

IJPCBS 2013, 3(4), 1191-1199 ISSN: 2249-9504

6.4 Review and literature of Centella asiatica:

1. Effect of Centella asiatica on cognition and oxidative stress in an intracerebroventricular streptozotocin model of Alzheimer's disease in rats.

Veerendra Kumar MH *et al* reported that Rats treated with C. asiatica showed a dose-dependent increase in cognitive behaviour in both paradigms. A significant decrease in MDA and an increase in glutathione and catalase levels were observed only in rats treated with 200 and 300 mg/kg C. asiatica. 5. The present findings indicate that an aqueous extract of C. asiatica is effective in preventing the cognitive deficits, as well as the oxidative stress, caused by i.c.v. STZ in rats.

Clin Exp Pharmacol Physiol. 2003 May-Jun;30 (5-6):336-42.

2. Centella asiatica screened for it’s for acetylcholinesterase inhibitory activity.

Mukherjee PK *et al* reported that the hydroalcoholic extract from Centella asiatica, Nardostachys jatamansi, Myristica fragrans, Evalvulus alsinoides inhibited 50% of AChE activity at concentrations of 100-150 microg/mL. Andrographis paniculata and Nelumbo nucifera extracts showed a weak inhibition of acetylcholinesterase with IC(50) values of 222.41 +/- 19.87 microg/mL and 185.55 +/- 21.24 microg/mL, respectively. Physostigmine was used as a standard and showed inhibition of acetylcholinesterase with an IC(50) value of 0.076 +/- 0.0042 microg/mL.

Phytother Res. 2007 Dec;21(12):1142-5.

3. **Centella asiatica treatment during postnatal period enhances learning and memory in mice.**

Rao SB *et al* reported that the performance of juvenile and young adult mice was significantly improved in radial arm maze and hole board tests, but locomotor activity did not show any change compared to control. Treatment resulted in increased acetylcholine esterase activity in the hippocampus. Dendritic arborization of hippocampal CA3 neurons was also increased in terms of intersections and branching points, both at one month and 6 months. Results of the present investigation show that treatment during postnatal developmental stage with Centella asiatica extract can influence the neuronal morphology and promote the higher brain function of juvenile and young adult mice.

Physiol Behav. 2005 Nov 15;86(4):449-57. Epub 2005 Oct 6.

4. **The effect of aqueous extract of Centella asiatica on learning and spatial memory in Alzheimerís disease animal model:**

A.H.Doulah *et al* reported that the aqueous extract of Centella asiatica had potential uses of the neuroprotective action in NBM lesioned rats induced dementia and an antioxidant mechanism is involved.

BioSciences RRBS, 8(6), 2014 [237-243]

5. **Effect of Centella asiatica on Anxiety and Oxidative stress markers and their correlation:**

   Tripathi A. S *et al* reported that the aqueous extract of CA showed good anxiolytic and antioxidant activity as compared to hydroalcoholic extract and a possibility of an antioxidant mechanism may be involved in it as combination of an antioxidant further increased the activity of extracts of CA.

   Journal of Pharmacy Research 2010, 3(10),2418-2420

6. **Effect of Centella asiatica on arsenic induced oxidative stress and metal distribution in rats:**

Gupta R *et al* reported that co-administration of Centella asiatica protects animals from arsenic-induced oxidative stress but exhibits no chelating property. Further studies are recommended for determining the effect of co-administration of Centella asiatica during chelation therapy with a thiol chelator.

J Appl Toxicol. 2006 May-Jun;26(3):213-22.

7. Flavonoid In Enhancing Memory Function:

M.Krishnaveni *et al* reported that in 1930's, Hungarian scientists Rusznyak and Szent-Gyorgi identified a substance from lemon peels that reduced capillary permeability. This substance was an effective treatment in purpura patients who were resistant to vitamin C therapy. They named this substance "vitamin P" (P for permeability). Later it was discovered that this "vitamin P" (or citrin) was not a single substance instead a mixture of flavonoids. Flavonoids are a group of naturally occurring compounds which are widely distributed in nature and are ubiquitous in vegetables, berries, and fruits and fortunately in chocolate too. Flavonoids are a large family of polyphenolic compounds synthesized by plants and provide much of the flavor and color to fruits and vegetables. More than 6000 different flavonoids have been identified. Citrus fruits such as lemon, orange, tangerine, grapefruit and dark chocolate which have plenty of flavonoids improves healthy blood flow. Chocolate improves the function of blood vessels, allowing them to dilate, thereby preventing the formation of potentially damaging clots. The MAPK and PI3 kinase pathways are known to be critical in controlling the morphological mechanisms behind memory storage in the hippocampus and cortex of the brain. Flavonoids have the potential to enhance memory and learning by activating kinases within these pathways. One way they act is by regulating proteins such as the cAMP response element-binding protein (CREB), which is involved in the expression of important genes linked to memory. For example, CREB is crucial for the production of neurotrophins - proteins responsible for neuronal survival, differentiation, and function. The aim of this review is to have a better understanding on the biological actions of flavonoids on enchancement of brain memory and learning.

Journal of Pharmacy Research 2012,5(7),3870-3874

6.5 Review and literature of *Curcumin:*

1. Neuroprotective effects of *curcumin*.

Cole GM *et al*reported that despite concerns about poor oral bioavailability, *curcumin* has at least 10 known neuroprotective actions and many of these might be realized in vivo. Indeed, accumulating cell culture and animal model data show that dietary *curcumin* is a strong candidate for use in the prevention or treatment of major disabling age-related neurodegenerative diseases like Alzheimer's, Parkinson's, and stroke. Promising results have already led to ongoing pilot clinical trials.

Adv Exp Med Biol. 2007;595:197-212.

2. **Confirmation of acute toxicity studies of *CURCUMIN*:**

Shankar TN *et al*performed the toxicity studies on turmeric (Curcuma longa): acute toxicity studies in rats, guineapigs & monkeys.

Indian J Exp Biol. 1980 Jan;18(1):73-5

3. **An Overview of *Curcumin* in Neurological Disorders**

S. K. Kulkarni *et al*reported that the present review attempts to discuss some of the potential protective role of *curcumin* in animal models of major depression, tardive dyskinesia and diabetic neuropathy. These studies call for well-planned clinical studies on *curcumin* for its potential use in neurological disorders.

Indian J Pharm Sci. 2010 Mar-Apr; 72(2): 149–154.

4. ***Curcumin* structure-function, bioavailability, and efficacy in models of neuroinflammation and Alzheimer's disease.**

Begum AN *et al*reported that tetrahydrocurcumin (TC) did reduce neuroinflammation and soluble Abeta, effects that may be attributable to limiting JNK-mediated transcription. Because of its favorable safety profile and the involvement of misfolded proteins, oxidative damage, and inflammation in multiple chronic degenerative diseases, these data relating *curcumin* dosing to the blood and tissue levels required for efficacy should help translation efforts from multiple successful preclinical models.

J Pharmacol Exp Ther. 2008 Jul; 326(1): 196–208.

5. **Curcumin inhibits experimental allergic encephalomyelitis by blocking IL-12 signaling through Janus kinase-STAT pathway in T lymphocytes.**

Natarajan C *et al*studied that the inhibition of Janus kinase-STAT pathway by *curcumin* resulted in a decrease in IL-12-induced T cell proliferation and Th1 differentiation. These findings highlight the fact that curcumin inhibits EAE by blocking IL-12 signaling in T cells and suggest its use in the treatment of MS and other Th1 cell-mediated inflammatory diseases.

J Immunol. 2002 Jun 15;168(12):6506-13.

6.6 REVIEW AND LITERATURE OF *QUERCETIN:*

1. **Confirmation of acute toxicity studies of QUERCETIN:**

Shu-Ting Chan *et al*Oral and Intraperitoneal Administration of Quercetin Decreased Lymphocyte DNA Damage and Plasma Lipid Peroxidation Induced by TSA In Vivo.

BioMed Research International Volume 2014 (2014), Article ID 580626, 9 pages

2. **Flavonoids and the CNS:**

Jäger AK*et al*reviewed that the flavonoids of several classes are inhibitors of monoamine oxidase A or B, thereby working as anti-depressants or to improve the conditions of Parkinson's patients. Flavanols, flavanones and anthocyanidins have protective effects preventing inflammatory processes leading to nerve injury. Flavonoids seem capable of influencing health and mood.

Molecules. 2011 Feb 10;16(2):1471-85. doi: 10.3390/molecules16021471.

3. **Analgesic, antiinflammatory and CNS depressant activities of sesquiterpenes and a flavonoid glycoside from Polygonum viscosum.**

Datta BK*et al*reported that all test compounds exhibited CNS depressant activity in open field test, all but viscoazulone showed analgesic activity in Eddy's hot plate test, all sesquiterpenes inhibited acetic acid induced abdominal writhing in mice, and all but viscoazucine and the flavonoid glycoside exhibited mild to moderate antiinflammatory effect on carrageenan induced rat paw edema.

Pharmazie. 2004 Mar;59(3):222-5.

4. **In vitro studies indicate that miquelianin (quercetin 3-O-beta-D-glucuronopyranoside) is able to reach the CNS from the small intestine.**

Juergenliemk G*et al*reported that porcine cell cultures of brain capillary endothelial cells were used as a model of the blood-brain barrier (bbb) and epithelial cells of the plexus chorioidei as a model of the blood-CSF barrier (bcb). Results indicate no active transport in one direction. Although moderate, the permeability coefficients (bbb: Pc = 1.34 +/- 0.05 x 10(-6) cm/sec; bcb: Pc = 2.0 +/- 0.33 x 10(-6) cm/sec) indicate the ability of miquelianin to cross both barriers to finally reach the CNS.

Planta Med. 2003 Nov;69(11):1013-7.

5. Antidepressant activity of quercetin, a bioflavonoid, in streptozotocin-induced diabetic mice.

Anjaneyulu M *et al* reported that streptozotocin-induced diabetic mice exhibited prolonged immobility duration during the test as compared with age-matched control mice. Quercetin dose-dependently reduced the immobility period in diabetic mice, and this effect was comparable to that of fluoxetine (5 mg/kg, i.p.) and imipramine (15 mg/kg, i.p.). Fluoxetine and imipramine significantly lowered the immobility time in naive mice also, but quercetin failed to induce any antidepressant activity in naive mice. The results of our preliminary study indicate that quercetin has the potential to be employed as a therapy for depression associated with diabetes.

J Med Food. 2003 Winter;6(4):391-5.

7. PLANT PROFILE:

7.1 Allium sativum

7.2 Benincasa hispida

7.3 Celastrus paniculatus

7.4 ***Centella asiatica***

7.5 ***Curcumin***

7.6 ***Quercetin***

7.1 Allium sativum:[33,35]

Fig no: 25

7.1.1 Botanical Classification:

Kingdom	-	Plantae – Plants
Subkingdom	-	Tracheobionta – Vascular plants
Superdivision	-	Spermatophyta – Seed plants
Division	-	Magnoliophyta – Flowering plants
Class	-	Liliopsida – Monocotyledons
Subclass	-	Liliidae
Order	-	Liliales
Family	-	Liliaceae – Lily family
Genus	-	Allium L. – onion
Species	-	Allium sativum L. – cultivated garlic

7.1.2 BOTANIC DESCRIPTION:

Bawang is a low herb, 30 to 60 centimeters high. True stem is much reduced. Bulbs are broadly ovoid, 2 to 4 centimeters in diameter, consisting of several, densely crowded, angular and truncated tubers. Leaves are linear and flat. Umbels are globose, many flowered. Sepals are oblong, greenish white, slightly tinged with purple. Stamens are not exerted from the perianth.

7.1.3 HABITAT:

Garlic is easy to grow and can be grown year-round in mild climates. While sexual propagation of garlic is possible, nearly all of the garlic in cultivation is propagated asexually, by planting individual cloves in the ground. In colder climates, cloves are planted in the autumn, about six weeks before the soil freezes, and harvested in late spring or early summer. The cloves must be planted deep enough to prevent freeze/thaw, which causes mold or white rot.

Garlic plants can be grown closely together, leaving enough space for the bulbs to mature, and are easily grown in containers of sufficient depth. Garlic does well in loose, dry, well-drained soils in sunny locations, and is hardy throughout USDA climate zones 4–9. When selecting garlic for planting, it is important to pick large bulbs from which to separate cloves. Large cloves, along with proper spacing in the planting bed, will also improve bulb size. Garlic plants prefer to grow in a soil with a high organic material content, but are capable of growing in a wide range of soil conditions and pH levels.

7.1.4 DISTRIBUTION:

- Extensively grown in India. Mainly in Ludhiana, Karnataka, Tamil Nadu, Andra Pradesh, U.P and Gujarat, Batangas, Nueva Ecija, Ilocos Norte, Mindoro, and Cotobato.
- A native of southern Europe.
- Now widely cultivated in most parts of the world.

7.1.5 OTHER NAMES:

English name	- garlic
Sanskrit/Indian name	- Lasuna
Tamil	- acanam
Telugu	- velluli
Kannada	- belluli
Malayalam	- vellulli

7.1.6 CONSTITUENTS:

Saponins; tannins; sulfurous compounds; prostaglandins; alkaloids; volatile oils; allicin (bulb), cysteine sulfoxides (alliin), glutamylcysteine peptides. Allicin, ajoenes and sulfides are degradation products of alliin.

Some of garlic's effect is attributed to alicin, its active ingredient, which is converted to ajoene, allyl sulfides and vinyldithiins.

Allicin (dially thiosulfinate or dially disulfide) is generated only when the garlic is crushed or cut, which activates the enzyme allinase which metabolizes alliin to allicin.

Aged garlic products lack allicin, but may have activity due to the presence of S-allycysteine. allyl disulfide, allypropyl disulfide; inulin; protein; myrosinase.

7.1.7USES:

Antibacterial, antifungal, antiparasitic**,** Anticandidal,Hypertension, Hyperlipidemia, Anti-oxidant**,** Hypocholesterolemic, Anti-cancer, Chemoprotective, Hepatoprotective, Hematologic Effects, Antidiabetic, Anti-Thrombotic Activity, Cardiovascular Benefits, Antimicrobial, Anti-Ulcer, Anthelmintic, Analgesic, Anti-Nociceptive, Immune System Enhancement, Virucidal, Antifibrinolytic, H. pylori Inhibition,antispasmodic.

7.2 Benincasa hispida:[33, 36]

Fig no: 26

7.2.1 Botanical Classification:

Kingdom	-	Plantae
Subkingdom	-	Tracheobionta – Vascular plants
Superdivision	-	Spermatophyta – Seed plants
Division	-	Magnoliophyta – Flowering plants
Class	-	Magnoliopsida – Dicotyledons
Subclass	-	Dilleniidae
Order	-	Violales
Family	-	Cucurbitaceae – Cucumber family
Genus	-	Benincasa Savi – benincasa
Species	-	Benincasa hispida (Thunb.)Cogn. – waxgourd

7.2.2 BOTANIC DESCRIPTION:

kondol is a rather coarse, wide-spreading, softly hairy, annual vine with branched tendrils reaching a length of 4 to 8 meters. Leaves are rounded or kidney-shaped, 10 to 25 centimeters diameter, 5- to 7-lobed, heart-shaped at the base. Peduncles are hairy, those of the males being 5 to 15 centimeters long and of the females much shorter. Flowers are large and yellow, with a densely hairy bell-shaped calyx tube. Petals are 5 and spreading, 3 to 5 centimeters long. Fruit is ellipsoid or ovoid, 25 to 40 centimeters long, with few to many fragile hairs, green, and densely covered with a white and waxy bloom. The seeds are many, oblong, and compressed.

7.2.3 HABITAT:

It is grown in well drained loam and sandy soils, in warm mild climates, but will not tolerate frosts. The crops are grown in riverbeds or furrows, and needs constant irrigation during the growing season. Ash Gourd is believed to have originated in Java, Indonesia. The Chinese have been cultivating it for over 2,000 years. Its medicinal uses first appeared in 659 AD in the Materia medica of the Tang dynasty. In Chinese medicine, the rind is used to treat urinary dysfunction and the seeds for vaginal discharge. The fruit is used to treat summer fevers.

In Ayurveda, the fruit is abeneficial for the management of a host of medical problems, including epilepsy, lung diseases, asthma, cough, urine retention and internal hemorrhage. It is also an excellent remedy for tapeworms.

7.2.4 DISTRIBUTION:

Native – Philippines, India, java, Japan, Malaya, Polynesia.

7.2.5 OTHER NAMES:

English name	- ash gourd, white gourd, winter gourd, tallow gourd
Sanskrit	- brihatphala, ghrinavasa, gramyakarkati
Tamil	- Neer poosani kai
Hindi name	- Petha, Pethakaddu
Bengali	- kumra, chalkumra
Telugu	- boodidha Gummadi kaaya

7.2.6 CONSTITUENTS:

- Amino acids, mucins, mineral salts, vitamins B and C, fixed oil, 44%; starch, 32%; an alkaline, cucurbitine; an acid resin; the proteids, myosin and vitellin; and sugar, 4%.
- Phytochemical studies indicate two triterpenes, alunsenol and mutiflorenol, with mast cell stabilizing effects in rats.
- Major constituents of the fruit are triterpenoids, flavonoids, glycosides, saccharides, carotenes, vitamins, ß-sitosterin, and uronic acid.
- Fruits yielded three new triterpenoids (3α,29-O-di-trans-cinnamoyl-D:C-friedooleana-7,9(11)-diene , oleanolic acid 28-O-β-d-xylopyranosyl-[β-d-xylopyranosyl-(1→4)]-(1→3)-α-l-rhamnopyranosyl-(1→2)-α-l-arabinopyranoside, and oleanolic acid 28-O-β-d-glucopyranosyl-(1→3)-β-d-xylopyranosyl-[β-d-xylopyranosyl-(1→4)]-(1→3)-α-l-

rhamnopyranosyl-(1→2)-α-l-arabinopyranoside), together with 12 known compounds (multiflorenol, isomultiflorenyl acetate, stigmasterol, stigmasterol3-O-β-d-glucopyranoside, α-spinasterol, α-spinasterol 3-O-β-d-glucopyranoside, β-sitosterol, daucosterol, arbutin, nicotinic acid, (+)-pinonesinol, and ethyl β-d-glucopyranoside).

7.2.7 USES:

Anxiolytic, antioxidant, astringent, Alzheimer's disease, aphrodisiac, anticompulsive effect, hepatoprotective, anthelmintic, analgesic, nootropic, anti-convulsant, to treat dementia, demulcent, diuretic, antifungal, anti-inflammatory, anti-ulcer, bronchodilator, anti-angiogenic effect. As a source of Vitamin B and C.In Ayurveda used for coughs, epilepsy, asthma, peptic ulcers. It is also the main ingredient in "Kusumanda Lehyam", used as tonic and for various conditions like epilepsy, constipation, hemorrhoids, dyspepsia, syphilis and diabetes. In India, used for treatment of peptic ulcer: Juice is squeezed out of grated gourd, equal amounts of water is added, taken daily on an empty stomach, with no food intake for 2 to 3 hours. Fruit juice is used for insanity, epilepsy.

7.2 Celastrus paniculatus:[33, 37]

Fig no: 27

7.3.1 BOTANICAL CLASSIFICATION:

Kingdom	-	Plantae – Plants
Subkingdom	-	Tracheobionta – Vascular plants
Superdivision	-	Spermatophyta – Seed plants
Division	-	Magnoliophyta – Flowering plants
Class	-	Magnoliopsida – Dicotyledons
Subclass	-	Rosidae

Order - Celastrales

Family - Celastraceae – Bittersweet family

Genus - Celastrus L. – bittersweet

Species - Celastrus paniculatus Willd. – Oriental bittersweet

7.3.2BOTANICDESCRIPTION:

Bilogo is a smooth woody vine, reaching a length of 4 to 10 meters. Branches are pendulous. Leaves are ovate to elliptic-ovate, 5 to 12 centimeters long, toothed at the margins. Flowers are numerous, greenish or greenish white, borne on lax, pendulous panicles, 7 to 18 centimeters long and about 5 millimeters in diameter. Fruit is ovoid or subglobose, 7 to 9 millimeters long, yellow, three-celled and usually three-seeded. Seeds are red and surrounded by a fleshy aril.

7.3.3 HABITAT:

Celastrus Paniculatus is a deciduous vine plant which grows natively throughout the Indian subcontinent. It has been used in various Indian cultural and medical traditions, such as Ayurveda and Indian Unani. The plant, which is widely known as the “intellect tree,” is used to both sharpen mental focus and relax the nerves.

7.3.4 DISTRIBUTION:

Native - Cagayan, Isabela, Ilocos Norte, Nueva Viscaya, Pangasinan, Bulacan, Bataan, Rizal, and Cavite Provinces in Luzon; and in Mindoro, Palawan, and Mindanao.

- Also occurs in India through Malaya to New Caledonia.

7.3.5 OTHER NAMES:

English name - Black Oil Plant

Sanskrit - Jyotishmati

Tamil - kuvarikuntal

Hindi - Mal-kangani

Bengal - kijri, malkangani

Telugu - kasara-tige, maneru

7.3.6 CONSTITUENTS:

- Seeds yield oil, a bitter resinous principle, tannin and ash, celastrine and paniculatin, oleum nigrum an empyreumatic black oil. Fatty oil contains colasterol and a coloring matter, chromagen. A study of the leaves suggested a small amount of scarcely poisonous alkaloid and a glucoside. Formic, acetic, benzoic, palmitic, stearic, oleic, linoleic, linolenic, palmitic acid, phytol, erucic acid, trans-beta-copaene, linalool, gallic acid, tannic acid.

7.3.7 USES:

Antioxidant, Nootropic effect, Learning and Memory, Analgesic, Anti-Inflammatory, Cognitive Enhancement, Neuroprotective, Antibacterial, Antifungal, Anthelmintic, Immunomodulatory Intestinal Relaxant, Hypolipidemic, Neuromodulating, Antidepressant, antirheumatic used for cases of paralysis, nerve-stimulant, brain tonic, aphrodisiac, appetizer, expectorant, liver tonic, to treat gout.

- Leaves used for dysentery.

- used as a powerful stimulant.

- In Ayurveda, bark considered as abortifacient; the leaves and leaf sap used as antidote to opium poisoning. Also used as brain tonic, appetite stimulant, and emetic.

- In Greco-Arabic Yunani medicine, seed oil used to treat physical weakness, mental confusion, asthma, headaches, joint pains and arthritis. Also used as a sexual stimulant.

- In India, used for memory difficulties, to improve memory recall and retention. Roots used for purifying the blood, eradicating stomach parasites; root paste for skin disease, ground roots for burns and boils; mixed with C. asiatica as an intellect enhancing tonic. Crush bark stem used as antidote to cobra venom; bark decoction used as abortifacient. Leaves used as anthelmintic.

7.4 Centella asiatica: [33, 38]

Fig no: 28

7.4.1 BOTANICAL CLASSIFICATION:

Kingdom	-	Plantae – Plants
Subkingdom	-	Tracheobionta – Vascular plants
Superdivision	-	Spermatophyta – Seed plants
Division	-	Magnoliophyta – Flowering plants
Class	-	Magnoliopsida – Dicotyledons
Subclass	-	Rosidae
Order	-	Apiales

Family - Apiaceae/Umbelliferae – Carrot family

Genus - Centella L. – centella

Species - Centella asiatica (L.)Urb. – spadeleaf

7.4.2 BOTANIC DESCRIPTION:

Takip-kohol is a prostrate, creeping, sparingly hairy or nearly smooth perennial herb, with delicate and slender stems rooting at the nodes. Leaves are rounded to reniform, 2 to 5 centimeters wide, horizontal, more or less cupped, rounded at the tip, and kidney-shaped or heart-shaped at the base, palmately veined, margins undulate-crenate, the rounded lobes often overlapping. Petioles are erect, 3 to 20 centimeters long. Flowers are dark-purple, axillary, ovate, and about 1 centimeter long. Peduncles occur in pairs or threes, less than 1 centimeter long and usually bear 3 sessile flowers. Fruits are minute, ovoid, white or green, and reticulate, each with 9 subsimilar longitudinal ridges. Carpels are five, cylindric compressed, about 2.5 millimeter long, white or green, reticulate. Ovary is inferior. Stamens are 5, epigynous.

7.4.3 HABITAT:

Centella asiatica is indigenous to the Indian subcontinent, Southeast Asia, and wetland regions of the Southeastern US. Because the plant is aquatic, it is especially sensitive to biological and chemical pollutants in the water, which may be absorbed into the plant. It can be cultivated in drier soils as long as they are watered regularly enough.

7.4.4 DISTRIBUTION:

Native – Philippines, india

7.4.5 OTHER NAMES:

English name : Indian Penny Wort

Sanskrit : Madukaparni

Tamil : Vallarai

Hindi name : Bengsag, Brahmi

Bengali name : Thulkudi

Telugu name : Sarasvathy Aku

7.4.6 CONSTITUENTS:

Asiaticoside, centelloside, madecassoside, brahmoside, brahminoside, thankuniside, sceffoleoside, centellose, asiactic-, brahmic-, centellic- and madecassic acids vellarine, carbohydrates, resins, proteins, ash, alkali, alkaline salts, phosphates, and tannins.

Triterpenoid glycosides, phytosterols, amino acids, free acids, volatile oils and flavonoids. Resinous, tannic acid and sugar, pectin and albuminous matter.

Triterpenoid saponins include asiaticoside, centelloside, madecassoside and asiatic acid. Alkaloids, flavonoids, tannins, terpenoids, saponin, steroids, proteins, reducing sugars, carbohydrates and cardiac glycosides.

Terpenic acetateß, ß-caryophyllene, farnesene, tans-ß-farnesene, gemacrene-D, α-humulene, bicylogermacrene, sesquiterpene and p-cymol.

7.4.7 USES:

Anxiolytic, Bactericidal, Wound Healing, Antioxidant, Immunomodulatory, Nerve Regeneration, Gastroprotective, Anti-Ulcer,Scleroderma, Periodontal Healing, Larvicidal, Diabetic Microangiopathy, Venous Insufficiency, Anti-Enteropathogens, Anti-Convulsant, Neuroprotective, Antibacterial, Improvement of Behavioral Deficits, Antibacterial, Anti-Diabetic, Neuroprotective, Improvement of Learning Disability, Anti-Inflammatory, Neurostimulant, Memory Improvement, Stimulation of Cell-Mediated Immune System, DNA Protective Effect, Antimicrobial, Antifungal, Anti-Carcinogenic, Ameliorating Effect on Learning and Memory Deficit, Anti-Proliferative, Diuretic Effect, Psoriasis.

7.5 *CURCUMIN*: [39]

7.5.1 CHEMICAL STRUCTURE:

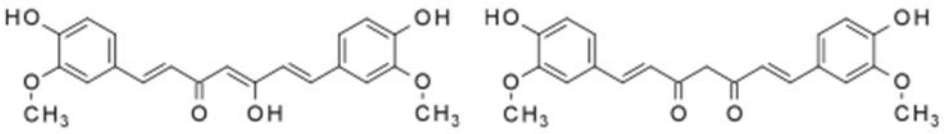


Fig no: 29

7.5.2 INTRODUCTION:

Curcumin is a bright yellow chemical produced by some plants. It is the principal curcuminoid of turmeric (Curcuma longa), a member of the ginger family (Zingiberaceae). It is sold as an herbal supplement, cosmetics ingredient, food flavoring, and food coloring. As a food additive, its E number is E100.

It was first isolated in 1815 when Vogel and Pierre Joseph Pelletier reported the isolation of a "yellow coloring-matter" from the rhizomes of turmeric and named it curcumin. Although curcumin has been used historically in Ayurvedic medicine, its potential for medicinal properties remains unproven and is questionable as a therapy when used orally.

Chemically, curcumin is a diarylheptanoid, belonging to the group of curcuminoids, which are natural phenols responsible for turmeric's yellow color. It is a tautomeric compound existing in enolic form in organic solvents and as a keto form in water.

7.5.3 CHEMISTRY:

Curcumin incorporates several functional groups whose structure was first identified in 1910. The aromatic ring systems, which are phenols, are connected by two α, β-unsaturated carbonyl groups. The diketones form stable enols and are readily deprotonated to form enolates; the α, β-unsaturated carbonyl group is a good Michael acceptor and undergoes nucleophilic addition.

Curcumin is used as a complexometric indicator for boron. It reacts with boric acid to form a red-colored compound, rosocyanine.

7.5.4 Pharmacology

Curcumin, which shows positive results in most drug discovery assays, is regarded as a false lead that medicinal chemists include among "pan-assay interference compounds" attracting undue experimental attention while failing to advance as viable therapeutic or drug

leads. In vitro, curcumin inhibits certain epigenetic enzymes (the histone deacetylases: HDAC1, HDAC3, HDAC8), transcriptional co-activator proteins (the p300 histone acetyltransferase) and the arachidonate 5-lipoxygenase enzyme.

7.5.5 TOXICITY:

Two preliminary clinical studies in cancer patients consuming high doses of curcumin (up to 8 grams per day for 3–4 months) showed no toxicity, though some subjects reported mild nausea or diarrhea.

7.5.6 APPLICATIONS:

The most common applications are as a dietary supplement, in cosmetics, as a food coloring, and as flavoring for foods such as turmeric-flavored beverages (Japan).

7.6 *QUERCETIN*: [40]

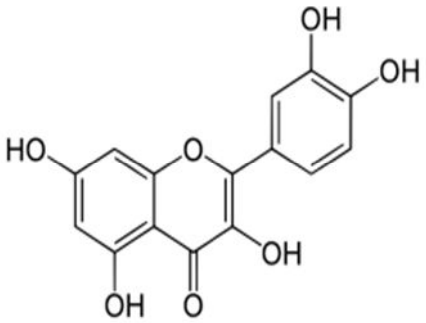


Fig no: 30

Quercetin is a plant polyphenol from the flavonoid group, found in many fruits, vegetables, leaves, and grains. It can be used as an ingredient in supplements, beverages, or foods.

7.1.1 OCCURRENCE

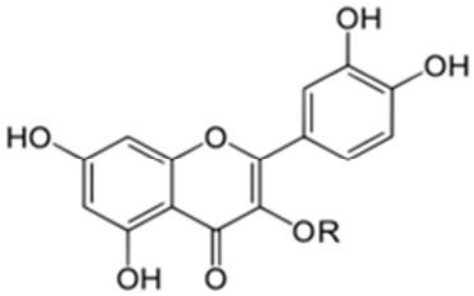
Quercetin is a flavonoid widely distributed in nature. The name has been used since 1857, and is derived from quercetum (oak forest), after Quercus. It is a naturally occurring polar auxin transport inhibitor. Quercetin is one of the most abundant dietary flavonoids with an average daily consumption of 25–50 mgs.

7.6.2 BIOSYNTHESIS

In plants, phenylalanine is converted to 4-coumaroyl-CoA in a series of steps known as the general phenylpropanoid pathway using phenylalanine ammonia-lyase, cinnamate-4-hydroxylase, and 4-coumaroyl-CoA-ligase. One molecule of 4-coumaroyl-CoA is added to three molecules of malonyl-CoA to form tetrahydroxychalcone using 7, 2′-dihydroxy-4′-methoxyisoflavanol synthase. Tetrahydroxychalcone is then converted into naringenin using chalcone isomerase.

Naringenin is converted into eriodictyol using flavanoid 3′-hydroxylase. Eriodictyol is then converted into dihydroquercetin with flavanone 3-hydroxylase, which is then converted into quercetin using flavonol synthase**.**

7.6.3 GLYCOSIDES



Glycoside	R
Hyperoside	galactosyl
Isoquercetin	glucosyl
Quercitrin	rhamnosyl
Rutin	rhamno-glucosyl

Fig no: 31 (3-O-Glycosides of quercetin)

Quercetin is the aglycone form of a number of other flavonoid glycosides, such as rutin and quercitrin, found in citrus fruit, buckwheat and onions. Quercetin forms the glycosides quercitrin and rutin together with rhamnose and rutinose, respectively. Likewise guaijaverin is the 3-O-arabinoside, hyperoside is the 3-O-galactoside, isoquercitin is the 3-O-glucoside and spiraeoside is the 4′-O-glucoside. CTN-986 is a quercetin derivative found in cottonseeds and cottonseed oil. Miquelianin is the quercetin 3-O-β-D-glucuronopyranoside.

7.6.4 RUTIN DEGRADATION PATHWAY

The enzyme quercitrinase can be found in Aspergillus flavus. This enzyme hydrolyzes the glycoside quercitrin to release quercetin and L-rhamnose. It is an enzyme in the rutin catabolic pathway.

7.6.5 PHARMACOLOGY

Pharmacokinetics

The bioavailability of quercetin in humans is low and highly variable (0-50%), and is rapidly cleared with an elimination half-life of 1–2 hours after ingesting quercetin foods or supplements. Following dietary ingestion, quercetin undergoes rapid and extensive metabolism that makes the biological effects presumed from in vitro studies unlikely to apply in vivo.

METABOLISM

In rats, quercetin did not undergo any significant phase I metabolism. In contrast, quercetin did undergo extensive phase II (conjugation) to produce metabolites that are more polar than the parent substance and hence are more rapidly excreted from the body. The meta-hydroxyl group of catechol is methylated by catechol-O-methyltransferase. Four of the five hydroxyl groups of quercetin are glucuronidated by UDP-glucuronosyltransferase. The exception is the 5-hydroxyl group of the flavonoid ring which generally does not undergo glucuronidation. The major metabolites of orally absorbed quercetin are quercetin-3-glucuronide, 3'-methylquercetin-3-glucuronide, and quercetin-3'-sulfate.

IN VITRO PHARMACOLOGY

Quercetin has been reported to inhibit the oxidation of other molecules and hence is classified as an antioxidant. Quercetin contains a polyphenolic chemical substructure that stops oxidation by acting as a scavenger of free radicals that are responsible for oxidative chain reactions.

Quercetin also activates or inhibits the activities of a number of proteins. For example, quercetin is a non-specific protein kinase enzyme inhibitor. Quercetin has also been reported to have estrogenic (female sex hormone like) activities by activating estrogen receptors. Quercetin activates both estrogen receptor alpha (ERα) and beta (ERβ) with binding IC50s of 1015 nM and 113 nM respectively. Hence quercetin is somewhat ERβ selective (9 fold) and is roughly two to three orders of magnitude less potent than the endogenous estrogenic hormone 17β-estradiol. In human breast cancer cell lines, quercetin has also been found to act as an agonist of the G protein-coupled estrogen receptor (GPER).

8. SCOPE OF THE WORK

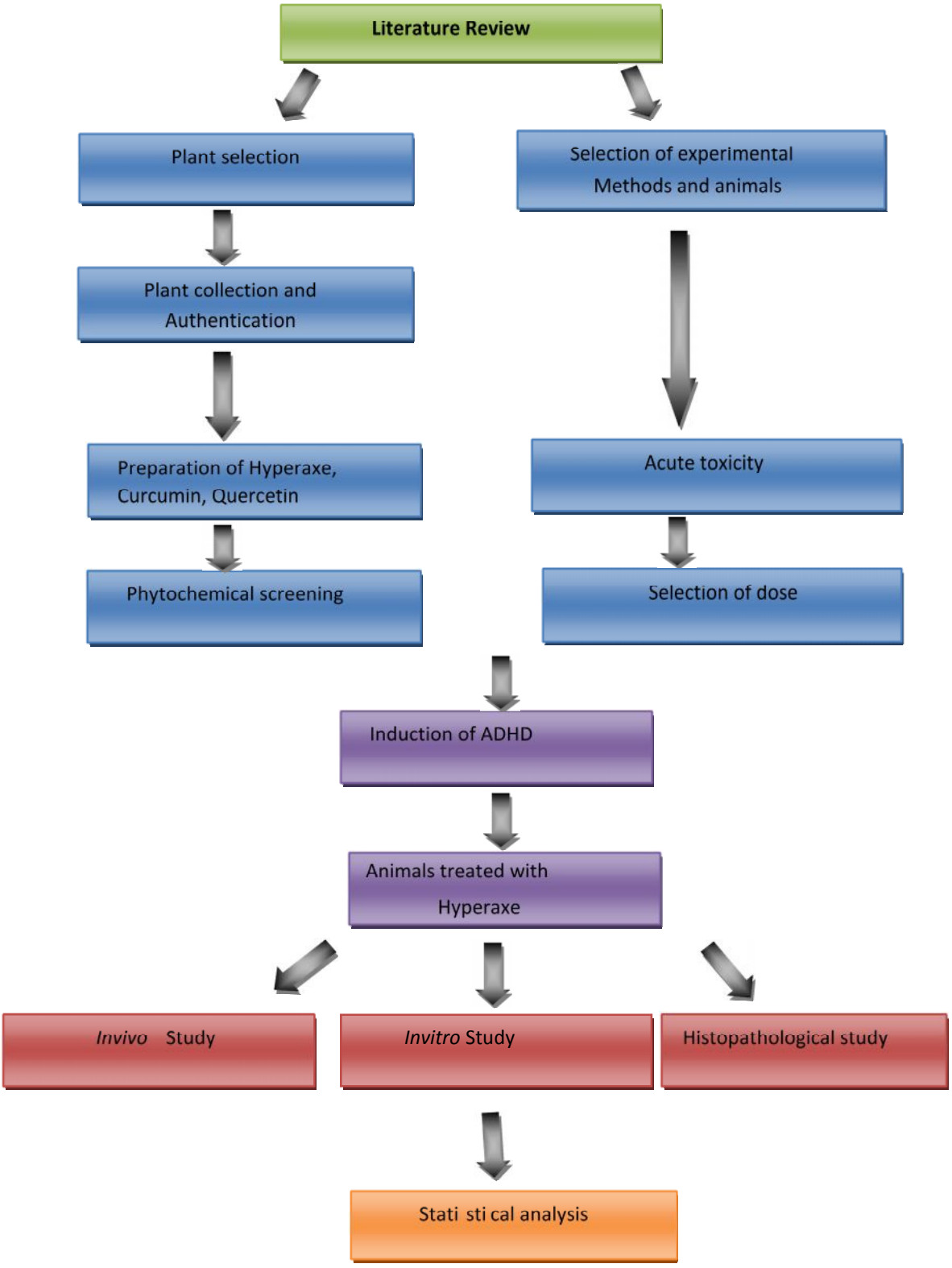
Attention Deficit Hyperactivity Disorder (ADHD) is a neurodevelopmental disorder that produces hyperactivity, inattention and impulsivity which causes difficulties in regular activities and impairs memory and causes problems around the surroundings due to unacceptable behavior. ADHD is characterized clinically by memory impairment, inattention and hyperactivity. An estimated 6.4 million with 4-17 years of age have ADHD in 2011-2012. By 2050, the number of children of age from 4-17 with ADHD may nearly triple, from 6.4 million to a projected 19.2 million.

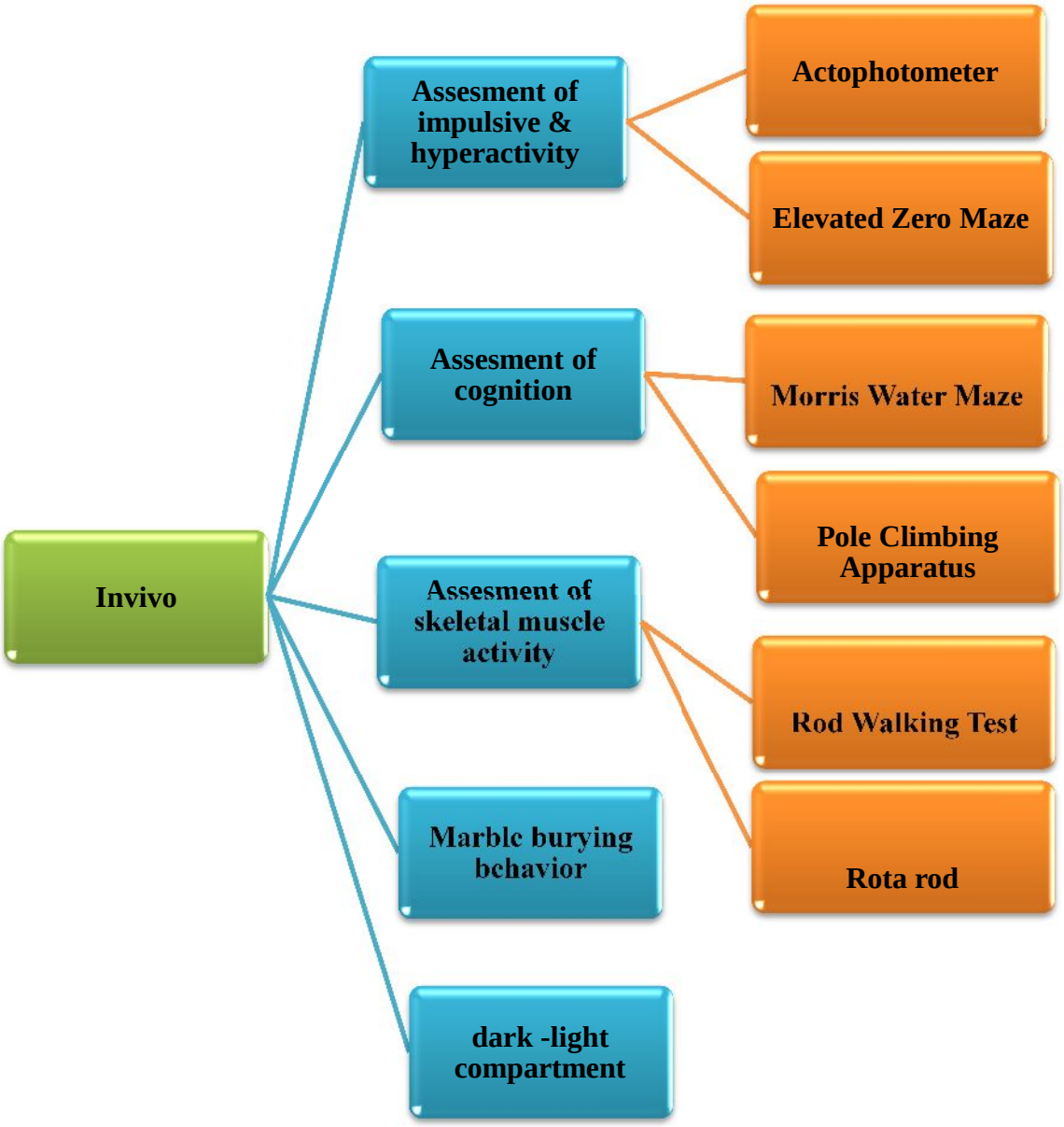
There is currently only symptomatic relief. But currently some category of drugs like stimulants and non-stimulants were used along with some antioxidants and some other supportive therapy. Therefore there is a lot of promising scope in the development of drug therapy for this chronic and debilitating disorder.

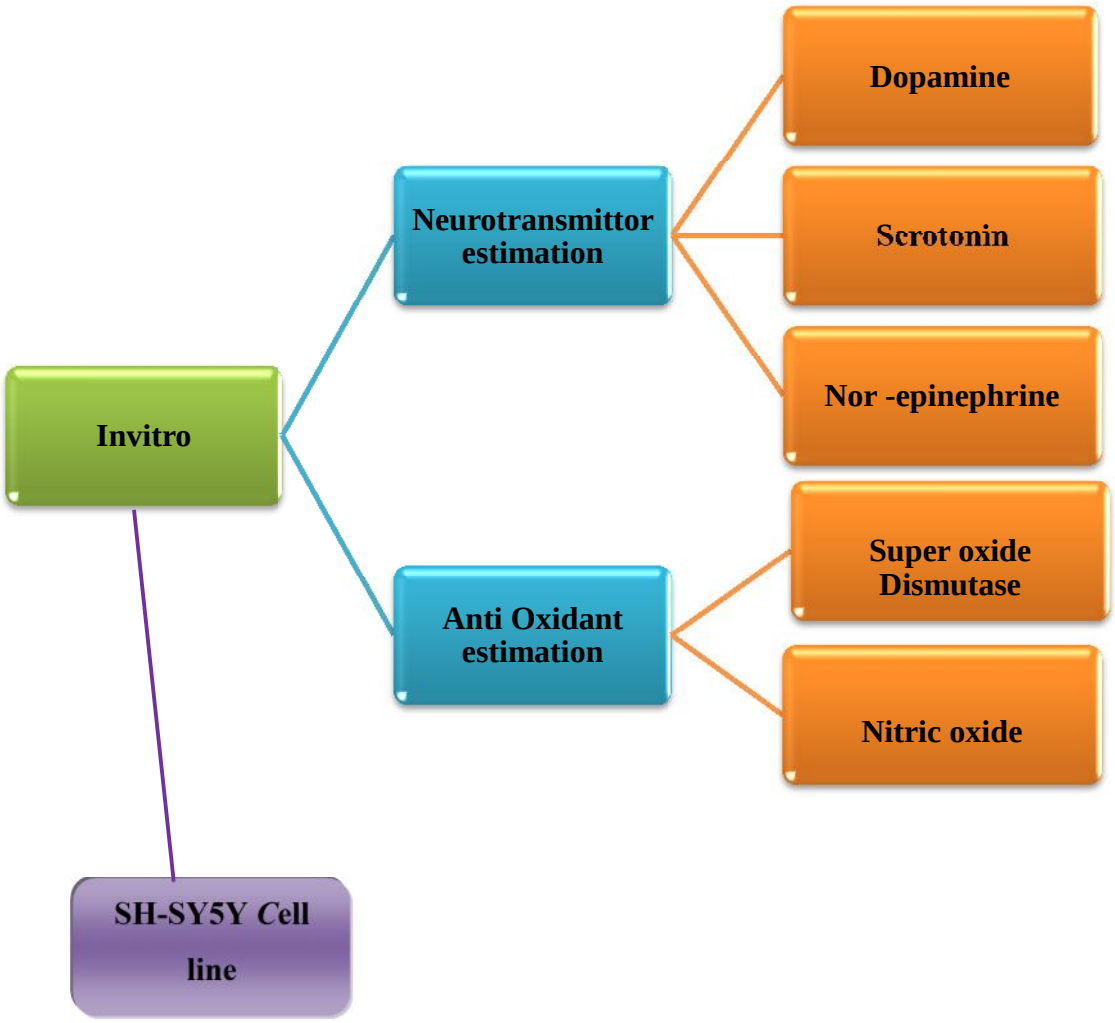
Since ancient time the herbal medicines are effective in the treatment of various ailment, many plants have been used in the treatment of several dreadful disease but they are not scientifically exploited or improperly used. And many plant based medicines are being used in Ayurveda, Siddha etc. for the treatment of ADHD and provide good results too. Therefore, these plant drugs deserve detailed studies in the light of modern science.

The present study is to prove the memory enhancement and cognitive effect of *hyperaxe, curcumin, quercetin* inADHD induced SD neonates using various memory retention experimentssuch as Elevated Zero maze, Morris water maze, Pole climbing apparatus, Marble burying behavior, Beam balance, etc.

9. PLAN OF WORK:




Invivo
Assesment of impulsive & hyperactivity
Actophotometer
Elevated Zero Maze
Assesment of cognition
Morris Water Maze
Pole Climbing Apparatus
Assesment of skeletal muscle activity
Rod Walking Test
Rota rod
Marble burying behavior
dark -light compartment


Dopamine
Neurotransmittor estimation
Scrotonin
Nor -epinephrine
Invitro
Super oxide Dismutase
Anti Oxidant estimation
Nitric oxide
SH-SY5Y Cell line

10. MATERIALS AND METHODS:

10.1 Collection, identification & authentication of plant materials:

- The seeds of Celastrus paniculatus were bought from amazon online shopping website.
- The leaves of Centella asiatica were purchased from local koyembedu market at Chennai.
- The fruits of Benincasa hispida were purchased from local koyembedu market.
- The cloves of Allium sativum were also purchased from local koyembedu market.

All the plant materials were identified and authenticated by Prof.P Jayaraman, Ph.D, Director, Institute of herbal botany, Plant anatomy and research centre, Chennai, Tamil Nadu, India.

10.2 METHOD AND PREPARATION OF EXTRACTS:

Fig no: 32

Fig no: 33

10.2.1 preparation of hydroalcoholic extract of *allium sativum*:

The fresh cloves of garlic were procured from the local market and the transparent covering of the cloves were removed manually and were sun dried for two weeks. After drying the dried garlic cloves were powdered in mixer. It was coarsely powdered. The powdered garlic was subjected to hot extraction in soxhlet apparatus using 60:40 ethanol:water as a solvent. The obtained extract was distilled and followed by evaporated to dryness to remove excess alcohol. The crude extract was stored at 0-4ºc for further analysis and to be used in the study.

10.2.2 preparation of hydroalcoholic extract of *benincasa hispida*:

The fruit was purchased from local market. The fruit was washed with running water to remove and the outer skin was peeled. The pulp was cut in to small pieces manually and was dried in hot air oven at 40-50ºc to remove the water content. The dried material was finely powdered using mixer and extracted in soxhlet apparatus using 60:40 ethanol:water as a solvent. The extracted was distilled and followed by evaporation to remove excess of solvent. The extract was stored at 0-4ºc for further study.

10.2.3 Preparation of hydroalcoholic extract of *Celastrus paniculatus*:

The seeds of celastrus paniculatus were purchased from amazon online shopping website,

India. The fresh seeds were finely powdered in a mixer and was subjected to hot extraction in soxhlet apparatus using 60:40 ethanol:water as a solvent. The extract was distilled and followed by evaporation to remove excess of solvent. The extract was stored at 0-4ºc for further study.

10.2.4 Preparation of hydroalcoholic extract of *Centella asiatica*:

The plant material was purchased from local market. The whole plant material was shade dried for a week and then oven dried at 40-50ºc for particular duration of time to remove excess moisture. The dried material was finely powdered in a mixer and was subjected for hot extraction in soxhlet apparatus using 60:40 ethanol:water as a solvent. The extract was distilled and followed by evaporation to remove excess of solvent. The extract was stored at 0-4ºc for further study.

10.3 PERCENTAGE YIELD:

The hydroalcoholic extract yielded dark brown, dark brown, brown yellow,dark green semisolid residues and percentage yield was found to be (20 % w/v).

10.4 PREPARATION OF *HYPERAXE*:

The extracts of *Allium sativum, Benincasa hispida, Centella asiatica, Celastrus paniculatus* were taken in equal amount (500mg:500mg:500mg:500mg) and mixed in geometric proportions. The combination of extract was dispersed in 0.5% Sodium caboxymethylcellulose. And it was named *hyperaxe.*

10.5 PREPARATION OF *CURCUMIN:*

0.2 parts of *Curcumin* was dispersed in 3 parts of Polyethylene glycol 400 and 1 part of distilled water and heated at 50-60ºc for 20 minutes to enhance its solubility followed by cooling to room temperature. The preparation was stirred well before administering to neonates through oral route. *Curcumin* preparation was stored at 10-15ºc.

10.6 PREPARATION OF *QUERCETIN:*

0.05 parts of *Quercetin* was dispersed in 5 parts of water. The preparation was stirred well before administering to neonates through oral route.*Quercetin* preparation was stored at 10-15ºc.

11. CHEMICALS USED:

S.NO	Chemical Name	Company Name
1	6-hydroxydopamine hydrogen bromide	Sigma aldrich
2	Acetic anhydride	Chem pure , Chennai
3	Alpha-napthol	SRL chemicals, sisco lab, Maharastra
4	Ammonium	SRL chemicals, sisco lab, Maharastra
5	Ascorbic acid	S.D. Fine- Chem Ltd, Mumbai
6	Atomoxetine Hcl	Sigma aldrich
7	Bromine	SRL Chemicals, Maharastra
8	Butanol	SRL Chemicals, Maharastra
9	Carbonate buffer	LobaChemie Pvt. Ltd, Mumbai
10	Copper acetate	SRL Chemicals, Maharastra
11	Copper sulphate	LobaChemie Pvt. Ltd, Mumbai
12	Curcumin	India mart
13	Diethyl ether	LobaChemie Pvt. Ltd, Mumbai
14	Distilled water	Andavar Distilled Water Company, Chennai
15	Epinephrine	SRL chemicals, sisco lab, Maharastra.
16	Ethanol	SRL chemicals, sisco lab, Maharastra.
17	Ferric chloride	S.D fine Chemicals, Mumbai
18	Ferric chloride	S.D fine Chemicals, Mumbai
19	Formaldehyde	S.D fine Chemicals, Mumbai
20	Glacial acetic acid	S.D fine Chemicals, Mumbai
21	Haematoxyli –fosin dye	S.D fine Chemicals, Mumbai
22	Heptane	S.D fine Chemicals, Mumbai
23	Hydrochloric acid	Ranbaxy Fine Chemical Ltd, NewDelhi
24	Imipramine Hcl	Sigma aldrich
25	Iodine	LobaChemie Pvt. Ltd, Mumbai
26	Iodine-potassium iodide solution	SRL chemicals, sisco lab, Maharastra

27	Iron and folic supplement	Amazon
28	Lead acetate	S.D fine Chemicals, Mumbai
29	Magnesium	S.D fine Chemicals, Mumbai
30	Magnesium and zinc supplement	Healthkart
31	Millon's reagent	S.D fine Chemicals, Mumbai
32	NaCl	S.D. Fine- Chem Ltd, Mumbai
33	Naphthyl ethylene diamine dihydrochloride	S.D. Fine- Chem Ltd, Mumbai
34	Omega-3 fatty acid supplement	Healthkart
35	O-phthalaldehyde reagent	S.D. Fine- Chem Ltd, Mumbai
36	Phosphoric acid	S.D. Fine- Chem Ltd, Mumbai
37	Picric acid	Paxy speciality chemicals, Chennai
38	Polyethylene glycol-400	Eisai Pharmaceuticals, Mumbai
39	Potassium bismuth iodide	LobaChemie Pvt. Ltd, Mumbai
40	Potassium chloride	LobaChemie Pvt. Ltd, Mumbai
41	Potassium hydroxide	LobaChemie Pvt. Ltd, Mumbai
42	Potassium mercuric iodine	Chemspure, Chennai
43	Potassium phosphate dibasic	Chemspure, Chennai
44	Potassium tartarate	LobaChemicals Pvt Ltd, Mumbai
45	Quercetin	Amazon
46	Sodium acetate	LobaChemie Pvt. Ltd, Mumbai
47	Sodium carbonate	Merck Specialties Pvt Ltd
48	Sodium carbonate	Merck Specialties Pvt Ltd
49	Sodium carboxy methyl cellulose	LobaChemie Pvt. Ltd, Mumbai
50	Sodium citrate	Chemspure, Chennai
51	Sodium EDTA	Chemspure, Chennai
52	Sodium hydrogen phosphate	Chemspure, Chennai
53	Sodium hydroxide	Chemspure, Chennai
54	Sodium nitro preside	Merck Specialties Pvt Ltd
55	Sodium phosphate dibasic	Merck Specialties Pvt Ltd

56	Sodium phosphate mono basic	Merck Specialties Pvt Ltd
57	Sodium thiosulphate	Merck Specialties Pvt Ltd
58	Sulfanilamide	Merck Specialties Pvt Ltd
59	Sulphuric acid	Chemspure, Chennai
60	Thionyl chloride	Chemspure, Chennai
61	Thiopentone sodium	Chemspure, Chennai
62	Xylene	Chemspure, Chennai

12. PRELIMINARY PHYTOCHEMICAL ANALYSIS: [41]

The Hydro-alcoholic extract of *Hyperaxe* was subjected to preliminary phytochemical screening for the presence or absence of phytoconstituents by the following methods.

1. TEST FOR ALKALOIDS:

The extract was treated with dilute hydrochloric acid and filtered. The filtrate is used in the following tests.

a) Mayer's reagent (Potassium Mercuric Iodine Solution)

0.5ml of the extract was treated with Mayer's reagent and the appearance of cream color indicates the presence of alkaloid

b) Dragendroff's test (Potassium Bismuth Iodide)

0.5ml of the extract was treated with Dragendroff's reagent and the appearance of reddish brown color precipitate indicates the presence of alkaloid.

c) Hager's test (Saturated solution of Picric acid)

0.5ml of the extract was treated with Hager's test and the appearance of yellow color precipitate indicates the presence of alkaloid.

d)Wagner's test (Iodine-Potassium Iodide Solution)

0.5ml of the extract was treated with Wagner's test and the appearance of brown color precipitate indicates the presence of alkaloid.

2. TEST FOR CARBOHYDRATES

a) Molisch's test:

The extract was treated with 3ml of alpha-napthol in alcohol and concentrated sulphuric acid was added along the sides of the test tube carefully. Formation of violet color ring at the junction of two liquids indicates the presence of carbohydrates.

b) Fehling's test (CuSO4.7H2O+KOH+Potassium Tartarate):

The extract was treated with Fehling's solution A and B heated in boiling water for few minutes. The appearance of reddish brown color precipitate indicates the presence of reducing sugars.

c) Benedict's test (Sodium citrate + sodium carbonate + CuSO4.7H2O)

The extract was treated with Benedict's test and heated in boiling water for few minutes. The appearance of reddish orange color precipitate indicates the presence of reducing sugars.

d) Barfoed's test (Copper Acetate+ Glacial acetic acid)

The extract was treated with Barfoed's test and heated in boiling water for few minutes. The appearance of reddish orange color precipitate indicates the presence of non-reducing sugars.

3. TEST FOR STEROIDS

a) LibermannBurchard test:

The extract was treated with small quantity of concentrated sulphuric acid, glacial acetic acid and acetic anhydride. The appearance of green color indicates the presence of steroids

4. TEST FOR PROTEINS

a) Biuret's test:

The extract was treated with copper sulphate and sodium hydroxide solution. The appearance of violet color indicates the presence of proteins.

b) Millon's test:

The extract was treated with Millon's reagent. The appearance of pink color indicates the presence of proteins.

5. TEST for Tannin's

a) The extract was treated with 10% lead acetate solution. The appearance of white precipitate indicates the presence of tannins.
b) The extract was treated with aqueous bromine solution. The appearance of white precipitate indicates the presence of tannins.

6. TEST FOR PHENOLS

a) The extract was treated with neutral ferric chloride solution. The appearance of violet indicates the presence of phenols.
b) The extract was treated with 10% sodium chloride solution. The appearance of cream color indicates the presence of phenols.

7. TEST FOR FLAVONOID'S

a) 5ml of extract solution was hydrolysed with 10%v/v sulphuric acid and cooled. Then, it is extracted with diethyl ether and divided into three portions in three separate test tubes. 1ml of diluted sodium carbonate, 1ml of 0.1N sodium hydroxide, and 1ml of strong ammonia solution were added to the first, second and third test tubes

respectively. In each test tube, development of yellow color demonstrated the presence of flavonoids.

b) **Shinoda's test**: The extracts were dissolved in alcohol, to that one piece of magnesium isadded followed by concentrated hydrochloric acid along the sides of the test tube drop wise. It is heated in a boiling water bath for few minutes. The appearance of magenta colour indicates the presence of flavonoids.

8. TEST FOR GUMS AND MUCILAGE

The extract was treated with 25ml of absolute alcohol and then solution was filtered. The filtrate was examined for its swelling properties.

9. TEST FOR GLYCOSIDES

The extract was dissolved in the glacial acetic acid and few drops of ferric chloride solution was added, followed by the addition of concentrated sulphuric acid, formation of red ring at the junction of two liquids indicates the presence of glycosides.

10. TEST FOR SAPONINS

1ml of the extract was diluted to 20ml with distilled water and shaken well in a test tube. The formation of foam in the upper part of the test tube indicates the presence of saponins.

11. TEST FOR TERPENES

The extract was treated with tin and thionyl chloride, appearance of pink color indicates the presence of terpenes.

12. TEST FOR STEROLS

The extract was treated with 5% potassium hydroxide solution; appearance of pink color indicates the presence of sterols.

13. ACUTE TOXICITY STUDIES: [42]

The acute toxicity was done 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.

13.1 Procedure:

Adult male Sprague dawley rats weighing 150-200 grams were used for the study. The starting dose level of 2000mg/kg body weight p.o of *Hyperaxe* 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 each rat 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 rat before and after administration of *hyperaxe* 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.

14. EXPERIMENTAL ANIMALS:

The Sprague dawley neonates weighing 20-30gm were used for this study. The neonates were procured from the animal house of C.L.BaidMetha college of pharmacy, Thoraipakkam, Chennai- 97. They were housed six per cage 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)

14.1 HUSBANDRY:

14.2 CONDITIONS:

Animals were housed under standard laboratory conditions, air conditioned with 12-15 filtered fresh air changes/hour. Environment: temperature 17-23°C, relative humidity 30-70%, with 12 hours fluorescent light (6.00 am to 6.00 pm) and 12 hours dark cycle.

14.3 HOUSING:

Rats were housed individually in standard polypropylene cages (size: approximately L 410 x B 280 x H 140 mm), with stainless steel top grill having facilities for pellet food and drinking water in glass bottle.

14.4 DIET AND WATER:

The control and negative control animals were provided with standard animal feed and water *adlibitium.*The animals were adapted to laboratory conditions one week prior to Initiations of experiments.

14.5 MODIFIED DIET FOR ADHD SD NEONATES:

1. Zinc and Magnesium -1 capsule per day
2. Iron and Folic acid – 1 capsule per day
3. Omega-3-Fatty acid – 1 capsule per day

All the capsules were opened manually and were triturated in a mortar with a pestle. The finely mixed food supplements were dispersed in drinking water for all groups except control and negative control. The diet was administered for 21 days i.e. until the experiment was terminated.

15. EXPERIMENTAL DESIGN:

Dose: 70µg in each lateral ventricle

15.1 INTRODUCTION:

- According to (shaywitz et al . , 1976; shaywitz et al . , 1978) on inducing 6-OHDA HBr through ICV of neonatal rats at PND5 disrupts the catecholamine system and has a significant effect on their neurotransmission and subsequent behavior.
- 6-OHDA is lethal to both dopaminergic and noradrenergic neurons and as a result it reduces DA/NA neurotransmission in the brain. So in this NA neurons can be protected by pretreatment with a NA reuptake inhibitor. (E.g. Desipramine, Robexitine, Atomoxetine, Bupropion).
- Behavioral consequences of 6-OHDA administration to neonatal rat pups results in temporary hyperactivity between PND12 and PND22 (shaywitz et al . , 1976) as well as learning and memory deficits (Archer et al . , 1988).

15.2 PROCEDURE:

- Sprague dawley litters were weaned for 4 days and caged at 22±1ºc under a 12 hour light and 12 hour dark cycle. They were provided free access to food and water ad libitum.
- At 5 days of age after birth neonates were pretreated with desipramine HCl (20mg/kg I.P) and after 1 hour 6-OHDA HBr (70µg in each lateral ventricle) was injected through intra cerebro ventricular (ICV) route to lesioned the dopaminergic neurons.

15.3 METHOD DEVELOPMENT:

15.4 PROCEDURE:

- Sprague dawley litters were weaned for 4 days and caged at 22±1ºc under a 12 hour light and 12 hour dark cycle. They were provided free access to food and water ad libitum.
- At 5 days of age after birth neonates were pretreated with Imipramine HCl (10mg/kg I.P) and after 1 hour 6-OHDA HBr (70µg in each lateral ventricle) was injected through intra cerebro ventricular (ICV) route to lesioned the dopaminergic neurons.

IAEC REFERENCE NO: IAEC/L/05/CLBMCP/2017

15.5 GROUPING:

S.no	Grouping	Treatment
1.	Group I	Control (0.5% Sodium Carboxymethylcellulose)
2.	Group II	6-OHDA lesioned rats (70µg in each lateral ventricle)
3.	Group III	6-OHDA lesioned rats+ Standard Atomoxetine HCl (1mg/kg p.o)
4.	Group IV	6-OHDA lesioned rats+ Atomoxetine HCl (1mg/kg p.o)+ Quercetin (30mg/kg p.o)
5.	Group V	6-OHDA lesioned rats+ low dose of *Hyperaxe* (200mg/kg, p.o)
6.	Group VI	6-OHDA lesioned rats+ high dose of *Hyperaxe* (400mg/kg, p.o)
7.	Group VII	6-OHDA lesioned rats+ low dose of *Curcumin* (200mg/kg, p.o)
8.	Group VIII	6-OHDA lesioned rats+ high dose of *Curcumin* (400mg/kg, p.o)

16. METHODS OF ASSESSMENT:

Invivo:

All the parameters were assessed on day 7, day 14 and day 21.

16.1 ASSESSMENT OF IMPULSIVE BEHAVIOR & HYPERACTIVITY:

i. Locomotor Activity Test:

1. Locomotor activity (horizontal activity) was measured using actimeter. Actophotometer which operates on photoelectric cells connected with a counter.
2. When a beam of light falling on the photocell is cut off by the animal a count is recorded and displayed digitally.
3. Each rat pup 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.
4. Actimeter is the combination of hole board and actophotometer in which both rats and mice can be placed.
5. Impulsive behavior was assessed on day 7, 14, 21.

ii. Elevated Zero Maze: [43,44]

1. Elevated zero maze is a modification of the elevated plus maze model of anxiety in rodents, which incorporates both traditional and novel ethological measures in the analysis of drug effects.
2. 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.
3. 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.
4. 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.
5. Impulsive behavior was assessed on day 7, 14, 21.

16.2 ASSESSMENT OF COGNITIVE BEHAVIOR:

iii. Morris water maze (MWM): [45, 46]

1. Behavioural tests were conducted using a Morris water maze (117 cm in diameter, and 50 cm high) in an experimental room.
2. The pool was filled with water to a depth of 40 cm and a temperature of 23 ± 2°c.
3. The water was made opaque with any suitable non-toxic ingredient to prevent the platform from being visible.
4. The position and orientation of the pool in the testing room remained unchanged throughout the study.
5. Four quadrants on the rim of the pool were designated as north (N), south (S), east (E) and west (W), thus dividing the pool into four quadrants (NW, NE, SE and SW).
6. A removable 8 cm escape platform was placed 2 cm below the water's surface in the centre of any one of the quadrants.
7. Cognitive function was assessed on day 7, 14, 21.

iv. Pole Climbing Test: [43, 47, 48]

1. Cook's Pole Climbing Apparatus use to study cognitive function, mainly a response to conditioned stimuli during learning & its retention.
2. The apparatus has an experimental chamber (25 × 25 × 25 cm) with the floor grid in a soundproof enclosure.
3. Scrambled shock (6mA) is delivered to the grid floor of the chamber composed of stainless steel rods.
4. A pole, 2.5 cm in diameter, hangs inside the chamber through a hole in the upper center of the chamber.
5. The study rat pup was placed in the chamber and allowed to explore the chamber for 60 seconds. Conditioned stimulus (CS) i.e buzzer signal was turned on and unconditioned stimulus (US) i.e. electric shock delivered through grid floor for 60 Sec.
6. Animal learned to associate the buzzer with the impending foot shock and was capable of avoiding the foot shock by climbing the pole after buzzer signal.
7. Avoidance response was defined as climbing reaction time <10 Sec only; and escape response was climbing after applying reaction time >10 Sec.

8. Every rat was subjected to maximum 05 trials on 1st day, and 24 hrs later, rat pup was subjected to relearning trials (2nd day 3 trials and on 3rd day one trial) and transfer latency was noted to check the retention of Conditioned Avoidance Response (CAR) and escape response.
9. Animals were screened by using this model and those who demonstrated at least one escape response either on day one or two were included in the study.
10. Cognitive function was assessed on day 7, 14, 21.

16.3 ASSESSMENT OF SKELETAL MUSCLE ACTIVITY:

v. Rod Walking Test: [43]

1. The ability of mice or rat to balance on a stationary, horizontal rod and walk on it from one end to the other of the rod measures cognitive study and learning activity.
2. Animals involved in the experiment were placed individually on the center of a rod (100 cm long, 5 mm in diameter, and positioned 23 cm above the table surface), parallel to it, and their latency to transfer from one end to another is recorded manually.
3. All rat pups were trained for five days and then tested for transfer latency.
4. Skeletal muscle activity was assessed on day 7, 14, 21.

vi. Rota rod: [49]

1. Animals are placed individually in separate lanes on rod rotating at 5rpm such that animals may walk forward to keep balance.
2. After 60s on rod, animals are returned to home cage and apparatus is cleaned with Virkon between trials.
3. Procedure is repeated for total of three trials separated by 10 min intertrial intervals.
4. Trial 3 may be repeated once if animal falls off rod before 60s cutoff, but no more than four trials should be run per animal.
5. Subjects must be able to stay on rod rotating at 5rpm for 60s before proceeding to testing. After providing the training to the animals they were used for experimental procedure.
6. The rotating rod is allowed to rotate at 25 rpm and animals from each group are placed individually on the rod and the fall off time was recorded for each animal.

7. Total 8 groups were involved and their average fall off time was concluded using MEAN±SEM.
8. Skeletal muscle activity was assessed on day 7, 14, 21.

16.4 OTHER PARAMETERS:

vii. Dark-Light Compartment: [50]

1. 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.
2. Rats 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.
3. All the cages containing rats are transferred to the behavior testing room 30 min before the first trial begins.
4. One chamber is brightly illuminated by white diodes (390 lux), whereas the other chamber is dark (2 lux).
5. Rats are placed into the dark side and the door is opened manually 3 seconds.
6. The door is used so that the rat 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.
7. Rats are allowed to move freely between the two chambers with door open for 10 min.
8. 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.
9. After each trial, all chambers are cleaned with super hypochlorous water to prevent a bias based on olfactory cues.
10. The activity was assessed on day 7, 14, 21.

viii. Marble Burying Behavior: [51]

1. Place one rat pup into a corner of the cage containing marbles, being careful to place the rat on bedding as far from marbles as possible, and place the filter-top cover on the cage.
2. Withhold food and water during the test. Allow rat pup to remain in the cage undisturbed for 30 min.

3. Remove the rat 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.
4. Score a marble as buried if two-thirds of its surface area is covered by bedding.
5. Average scores for the number of marbles buried for each rat pup was recorded manually. Retrieve all 20 marbles from the bedding. Dispose of bedding.
6. The activity was assessed on day 7, 14, 21.

17. NEUROTRANSMITTERS ESTIMATION: [52]

17.1 PREPARATION OF TISSUE EXTRACTS:

Reagents

1. HCl-butanol
2. Heptanes
3. 0.1M HCl: (0.85ml conc. HClupto 100ml of water)

Procedure

On the day of experiment SD neonates 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.

17.2 ESTIMATION OF DOPAMINE:

Reagents:

1. 0.4M HCl: 3.4ml conc. HClupto 100ml water
2. Sodium acetate buffer pH(6.9)
3. 5M NaOH
4. 0.1M Iodine solution (in ethanol)
5. Sodiumthiosulphate
6. 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.

$$\text{Unknown conc} = \frac{\text{intensity of unknown} * \text{conc of std}}{\text{intensity of std}}$$

17.3 ESTIMATION OF SEROTONIN:

The serotonin content was estimated by the method of Schlumpf.

Reagents

1. 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.

$$\text{Unknown conc} = \frac{\text{intensity of unknown} * \text{conc of std}}{\text{intensity of std}}$$

17.4 ESTIMATION OF NOREPINEPHRINE:

Reagents

1. 0.4M HCl: 3.4ml conc. HCl upto 100ml water
2. Sodium acetate buffer pH(6.9)
3. 5M NaOH:
4. 0.1M Iodine solution(in ethanol)
5. Sodiumthiosulphate
6. 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.

18. ESTIMATION OF ANTIOXIDANT ENZYME

18.1 ESTIMATION OF SUPEROXIDE DISMUTASE (SOD) [53]

Reagents

1. Carbonate buffer (100mM, pH 10.2)
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

18.2 In vitro nitric oxide radical (NO) scavenging assay: [54]

Reagents:

1. Sodium nitroprusside (SNP)
2. Phosphate buffered saline (pH 7.3)
3. Griess reagent

NO generated from sodium nitroprusside (SNP) was measured according to the method of Marcocci et al. (1994). Briefly, the reaction mixture (5.0 ml) containing SNP (5 mM) in phosphatebuffered saline (pH 7.3), with or without the plant extract at different concentrations, was incubated at 25˚C for 180 min in front of a visible polychromatic light source (25W tungsten lamp). The NO radical thus generated interacted with oxygen to produce the nitrite ion (NO) which was assayed at 30 min intervals by mixing 1.0 ml of incubation mixture with an equal amount of Griess reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% naphthylethylenediaminedihydrochloride). The absorbance of the chromophore (purple azo dye) formed during the diazotisation of nitrite ions with sulphanilamide and subsequent coupling with naphthylethylenediaminedihydrochloride was measured at 546 nm. The nitrite generated in the presence or absence of the plant extract was estimated using a standard curve based on sodium nitrite solutions of known concentrations.

19. In vitro cell line: [55, 56]

Cell line and culture:

SH-SY5Y cell line was obtained from NCCS, Pune. The cells were maintained in Minimal Essential-F12 Medium supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 5% CO_2 at 37 °C.

Reagents:

MEM –F12 was purchased from Hi Media Laboratories, Fetal Bovine Serum (FBS) was purchased from Cistron laboratories Trypsin, Water-soluble tetrazolium salt (WST-8) and Dimethyl sulfoxide (DMSO) were purchased from (Sisco research laboratory chemicals Mumbai). All of other chemicals and reagents were obtained from Sigma Aldrich Mumbai.

***In Vitro* assay for MTT activity (Mossman 1983)**

Cells (1 × 105/well) were plated in 24-well plates and incubated in 37^0C with 5% CO2 condition. After the cell reaches the confluence, the sample was added and incubated for 24hrs. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) or MEM-F12 without serum. 100µl/well (5mg/ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl--tetrazolium bromide (MTT) was added and incubated for 4 hours. After incubation, 1ml of DMSO was added in all the wells .The absorbance at 570 nm was measured with UV- Spectrophotometer using DMSO as the blank. Measurements were performed and the concentration required for a 50% inhibition (IC50) was determined graphically. The % cell viability was calculated using the following formula:

Calculation:

% cell viability = A570 of treated cells / A570 of control cells × 100

20. METHODS FOR HISTOPATHOLOGICAL STUDY:

The rat from each group were anaesthetized using intraperitoneal injection of thiopentone sodium. The brain was carefully removed without any injury after opening the skull. The collected brain was washed with ice cold normal saline and fixed in 10% formalin saline. Paraffin embedded sections were taken 100µm thickness and processed in alcohol-xylene series and stained with Haematoxyli-Eosin dye. The sections were examined microscopically for histopathological changes in the hippocampal zone.

21. 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

22. TABLES AND GRAPH:

TABLE NO: 2 Phytochemical screening of *Hyperaxe*

S.no	Constituents	Plant Extracts			
		Allium Sativum	***Benincasa Hispida***	***Celastrus Paniculatus***	***Centella Asiatica***
1	Alkaloids	+	+	+	+
2	Carbohydrates	+	+	-	+
3	Protein	+	+	-	+
4	Steroids	+	+	+	+
5	Phenols	+	+	-	+
6	Tannins	+	+	+	+
7	Flavanoids	+	+	+	+
8	Gums & Mucilage	-	+	-	-
9	Glycosides	+	+	-	+
10	Saponins	+	+	+	-
11	Terpenes	+	+	+	+
12	Sterols	-	+	+	-

Table no: 3 Effect of *Hyperaxe* in acute toxicity studies:

S. No	Treatment	Dose	Weight of animal (g)		Signs of Toxicity	Onset of Toxicity	Reversible or Irreversible	Duration
			Before test	After test				
1.	HYPERAXE	2000mg/kg	150	153	No signs of toxicity	Nil	Nil	14 days
2.	HYPERAXE	2000mg/kg	190	190				
3.	HYPERAXE	2000mg/kg	200	200				

Table no: 4 Effect of *Hyperaxe, Curcumin, Quercetin* in Actophotometer:

S.no	Groups	Activity Scores		
		Day 7	Day 14	Day 21
1	Control	128.16±0.1	267.33±0.1	468.5±0.02
2	Negative control	642.33±0.12	1118.83±0.1	1586.5±0.03
3	ATOMOXETINE	333.16±0.01	333.5±0.01	282.16±0.3
4	ATOMOXETINE+QUERCETIN	416.5±0.01^{a**b**}	351.33±0.03^{a**b**}	290.33±0.2^{a**b***}
5	HYPERAXE LOW DOSE	507.33±0.01^{a**b**}	456±0.1 ^{a**b**}	519.83±0.01^{a**b**}
6	HYPERAXE HIGH DOSE	444.66±0.02^{a**b**}	355±0.04^{a**b**}	304.16±0.1 ^{a**b***}
7	CURCUMIN LOW DOSE	530±0.01 ^{a**b**}	460±0.05^{a**b**}	486.33±0.3^{a**b**}
8	CURCUMIN HIGH DOSE	449.5±0.02 ^{a**b**}	358.83±0.04^{a**b**}	300.16±0.1^{a**b**}

Values are represented in Mean ± SEM, n=6
Comparison: a- Group II vs Group IV,V,VI,VII,VIII b- Group III vs Group IV,V,VI,VII,VIII 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, ****p<0.0001

Graph no: 1 Effect of *Hyperaxe, Curcumin, Quercetin* in Actophotometer:

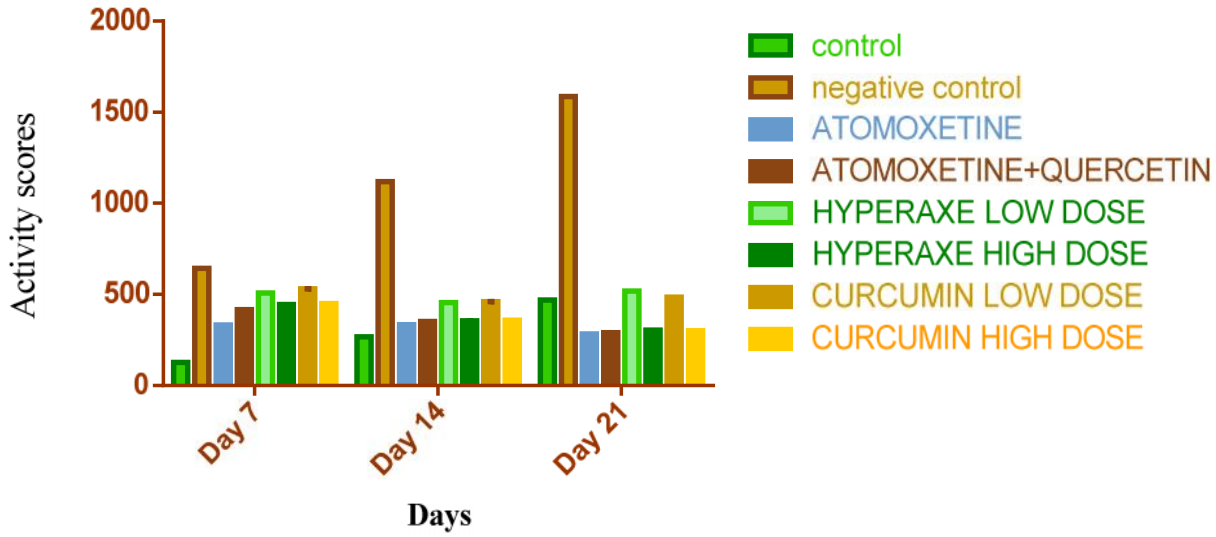


Table no: 5 Effect of *Hyperaxe, Curcumin, Quercetin* in Elevated Zero Maze:

S.no	Groups	% Alterations in open arm		
		Day 7	Day 14	Day 21
1	Control	26.16±0.02	27±0.1	27.3±0.1
2	Negative control	45.6±0.03	44.5±0.12	40.3±0.1
3	ATOMOXETINE	45.3±0.3	30.8±0.01	21.3±0.01
4	ATOMOXETINE+QUERCETIN	25±0.2 ^{a***b**}	17.6±0.01 ^{a**b**}	7.3±0.03 ^{a**b***}
5	HYPERAXE LOW DOSE	36±0.01 ^{a**b**}	30.16±0.01 ^{a**b**}	19.3±0.1 ^{a**b**}
6	HYPERAXE HIGH DOSE	35.8±0.1 ^{a**b**}	23±0.02 ^{a**b**}	17.8±0.04 ^{a**b***}
7	CURCUMIN LOW DOSE	32.3±0.3 ^{a**b**}	22.16±0.01 ^{a**b**}	15.8±0.05 ^{a**b**}
8	CURCUMIN HIGH DOSE	22.3±0.1 ^{a***b**}	16.3±0.02 ^{a**b**}	6.8±0.04 ^{a**b**}

Values are represented in Mean ± SEM, n=6
Comparison: a- Group II vs Group IV,V,VI,VII,VIII b- Group III vs Group IV,V,VI,VII,VIII 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, ****p<0.0001

Graph no: 2 Effect of *Hyperaxe, Curcumin, Quercetin* in Elevated Zero Maze:

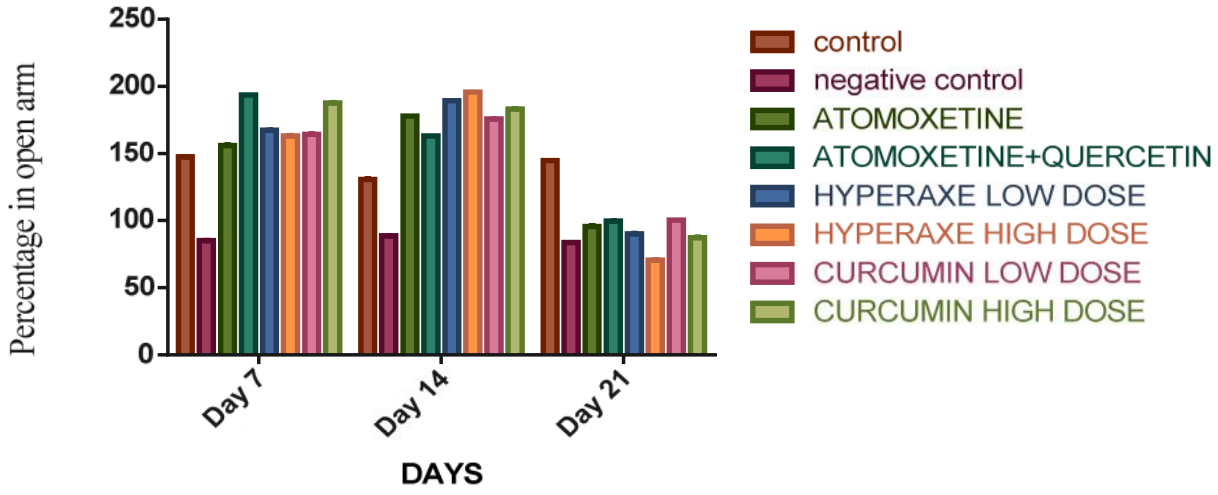


Table no: 6 Effect of *Hyperaxe, Curcumin, Quercetin* in Morris water maze:

S.no	Groups	Escape latency (sec)		
		Day 7	Day 14	Day 21
1	Control	60±0.02	54.3±0.1	52.5±0.1
2	Negative control	60±0.03	57±0.12	49.5±0.1
3	ATOMOXETINE	59±0.01	32.8±0.01	9.8±0.1
4	ATOMOXETINE+QUERCETIN	58.3±0.1 a**b**	33.5±0.02 a**b**	7.6±0.04 a**b***
5	HYPERAXE LOW DOSE	51.3±0.3 a**b**	17.3±0.01 a**b**	8.16±0.05 a**b**
6	HYPERAXE HIGH DOSE	16.5±0.1 a**b**	6±0.02 a**b**	4.3±0.04 a**b***
7	CURCUMIN LOW DOSE	52±0.3 a**b**	33±0.01 a**b**	11.16±0.01 a**b**
8	CURCUMIN HIGH DOSE	41.8±0.2 a**b**	11.4±0.01 a**b**	6±0.03 a**b**

Values are represented in Mean ± SEM, n=6
Comparison: a- Group II vs Group IV,V,VI,VII,VIII b- Group III vs Group IV,V,VI,VII,VIII 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, ****p<0.0001

Graph no: 3 Effect of *Hyperaxe, Curcumin, Quercetin* in Morris water maze:

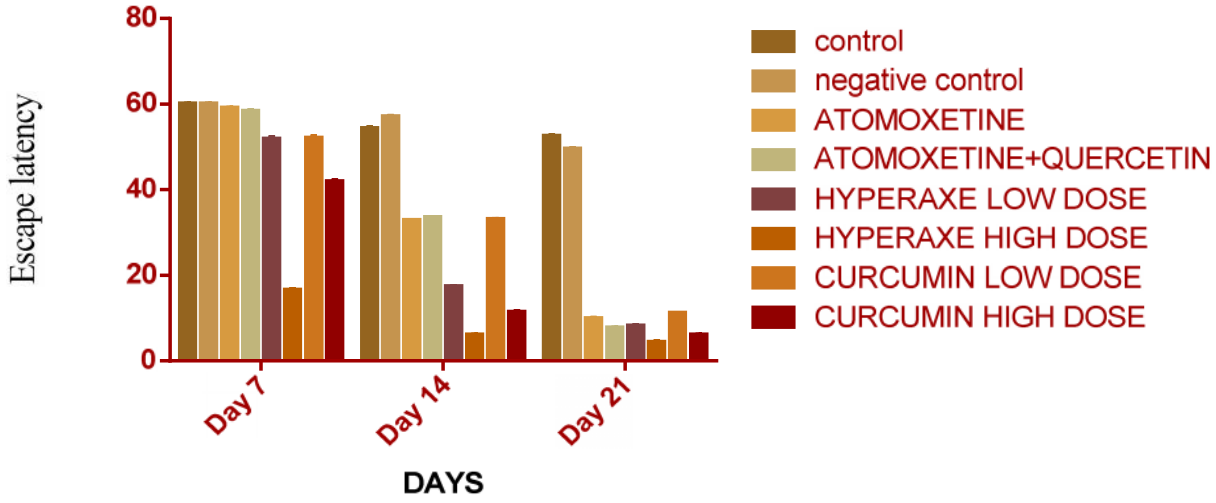


Table no: 7 Effect of *Hyperaxe, Curcumin, Quercetin* in Pole Climbing test:

S.no	Groups	Escape latency (sec)		
		Day 7	Day 14	Day 21
1	Control	60±0.02	53±0.1	24.6±0.1
2	Negative control	60±0.03	56.6±0.1	23.3±0.12
3	ATOMOXETINE	59.83±0.3	7.3±0.01	3±0.01
4	ATOMOXETINE+QUERCETIN	59.6±0.2 a**b**	5.8±0.03 a***b**	3±0.01 a**b***
5	HYPERAXE LOW DOSE	54.16±0.01 a**b**	16.6±0.1 a**b**	4.83±0.01 a**b**
6	HYPERAXE HIGH DOSE	45.16±0.1 a***b**	2.6±0.04 a**b**	2.5±0.02 a**b***
7	CURCUMIN LOW DOSE	53.83±0.3 a**b**	12.5±0.05 a**b**	4.8±0.01 a**b**
8	CURCUMIN HIGH DOSE	46.5±0.1 a**b**	4.16±0.04 a**b**	3±0.02 a**b**

Values are represented in Mean ± SEM, n=6
Comparison a- Group II vs Group IV,V,VI,VII,VIII b- Group III vs Group IV,V,VI,VII,VIII 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, ****p<0.0001

Graph no: 4 Effect of *Hyperaxe, Curcumin, Quercetin* in Pole Climbing test:

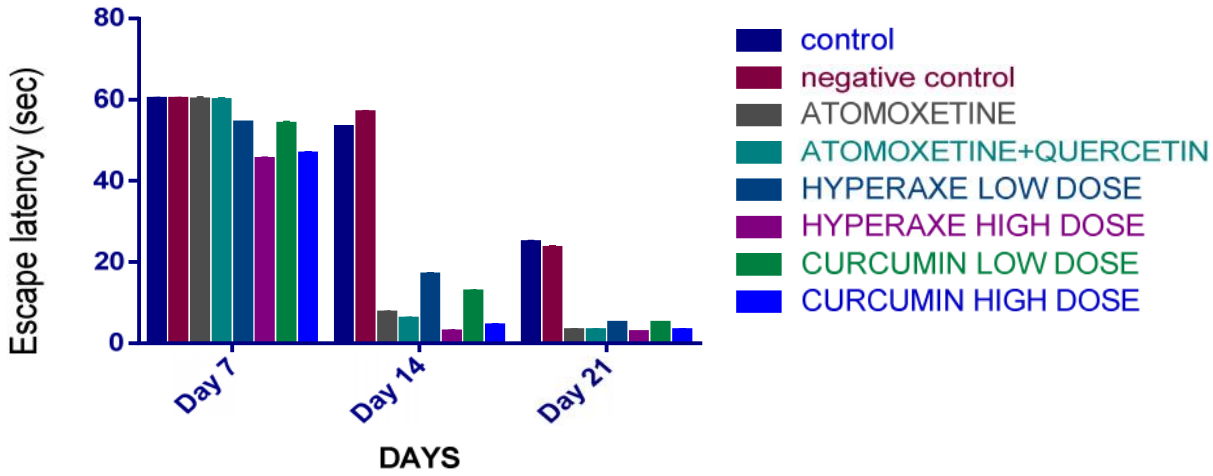


Table no: 8 Effect of *Hyperaxe, Curcumin, Quercetin* in Rod walking test:

S.no	Groups	% Alterations in open arm		
		Day 7	Day 14	Day 21
1	Control	26.16±0.02	27±0.1	27.3±0.1
2	Negative control	45.6±0.03	44.5±0.12	40.3±0.1
3	ATOMOXETINE	45.3±0.3	30.83±0.01	21.33±0.01
4	ATOMOXETINE+QUERCETIN	25±0.2 a**b**	17.66±0.01 a***b**	7.33±0.03 a**b***
5	HYPERAXE LOW DOSE	36±0.01 a*b**	30.16±0.01 a**b**	19.33±0.1 a**b**
6	HYPERAXE HIGH DOSE	35.83±0.1 a**b**	23±0.02 a*b**	17.83±0.04 a**b***
7	CURCUMIN LOW DOSE	32.3±0.3 a*b*	22.16±0.01 a*b*	15.83±0.05 a*b*
8	CURCUMIN HIGH DOSE	22.3±0.1 a**b**	16.3±0.02 a**b**	6.83±0.04 a**b***

Values are represented in Mean ± SEM, n=6
Comparison: a- Group II vs Group IV,V,VI,VII,VIII b- Group III vs Group IV,V,VI,VII,VIII 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, ****p<0.0001

Graph no: 5 Effect of *Hyperaxe, Curcumin, Quercetin* in Rod walking test:

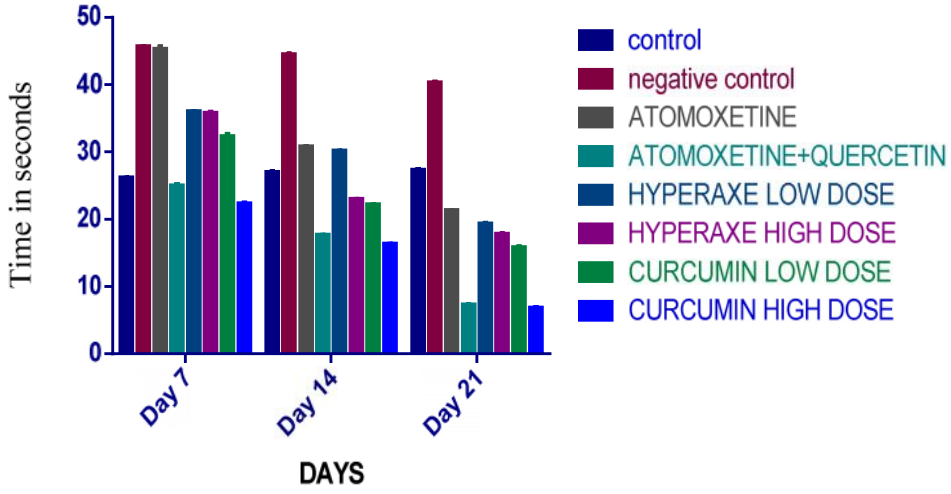


Table no: 9 Effect of *Hyperaxe, Curcumin, Quercetin* in Rota rod:

S.no	Groups	Fall off time		
		Day 7	Day 14	Day 21
1	Control	12±0.1	13±0.1	23±0.02
2	Negative control	576.16±0.12	870.16±0.1	1014±0.03
3	ATOMOXETINE	519.83±0.01	105.8±0.01	62.8±0.3
4	ATOMOXETINE+QUERCETIN	548.83±0.01^{a*b*}	217.8±0.03^{a**b**}	84.16±0.2 ^{a**b***}
5	HYPERAXE LOW DOSE	98.66±0.01 ^{a**b**}	61.6±0.1 ^{a**b**}	34.16±0.01 ^{a**b**}
6	HYPERAXE HIGH DOSE	17.83±0.02 ^{a**b**}	13±0.04 ^{a**b**}	8.83±0.1 ^{a**b***}
7	CURCUMIN LOW DOSE	271.5±0.01 ^{a**b**}	196±0.05 ^{a**b**}	111.16±0.3 ^{a**b**}
8	CURCUMIN HIGH DOSE	180.83±0.02^{a**b**}	80.16±0.04^{a**b**}	31.5±0.1 ^{a**b**}

Values are represented in Mean ± SEM, n=6
Comparison a- Group II vs Group IV,V,VI,VII,VIII b- Group III vs Group IV,V,VI,VII,VIII 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, ****p<0.0001

Graph no: 6 Effect of *Hyperaxe, Curcumin, Quercetin* in Rota rod:

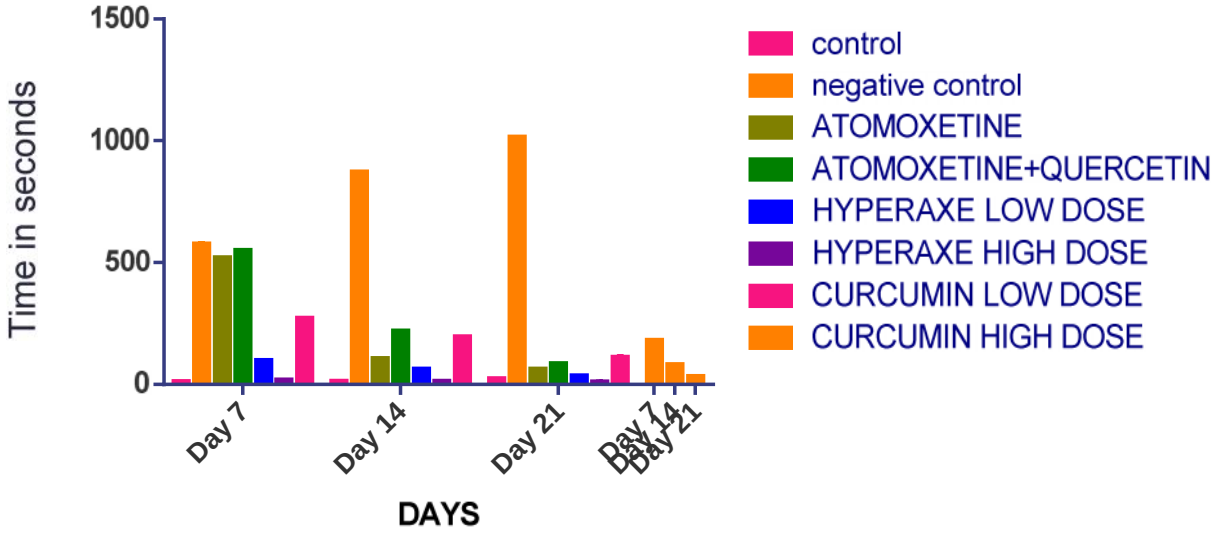


Table no: 10 Effect of *Hyperaxe, Curcumin, Quercetin* in Dark-Light Compartment:

S.no	Groups	Time spent in Light Compartment		
		Day 7	Day 14	Day 21
1	Control	130.8±0.1	144.7±0.02	147.5±0.04
2	Negative control	83.6±0.03	85±0.07	88.7±0.1
3	ATOMOXETINE	95.8±0.3	157.4±0.1	240±0.05
4	ATOMOXETINE+QUERCETIN	99.6±0.1 a**b**	135.5±0.04 a**b**	210±0.04 a**b***
5	HYPERAXE LOW DOSE	90.1±0.3 a*b*	140.2±0.04 a**b**	181.2±0.01 a**b**
6	HYPERAXE HIGH DOSE	70.5±0.2 a*b*	147.3±0.07 a***b**	220±0.03 a**b**
7	CURCUMIN LOW DOSE	100.5±0.01 a*b*	138.5±0.2 a**b**	175.65±0.1 a**b**
8	CURCUMIN HIGH DOSE	87.4±0.1 a*b**	143.5±0.3 a*b**	198.2±0.04 a**b**

Values are represented in Mean ± SEM, n=6
Comparison: a- Group II vs Group IV,V,VI,VII,VIII b- Group III vs Group IV,V,VI,VII,VIII 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, ****p<0.0001

Graph no: 7 Effect of *Hyperaxe, Curcumin, Quercetin* in Dark-Light Compartment:

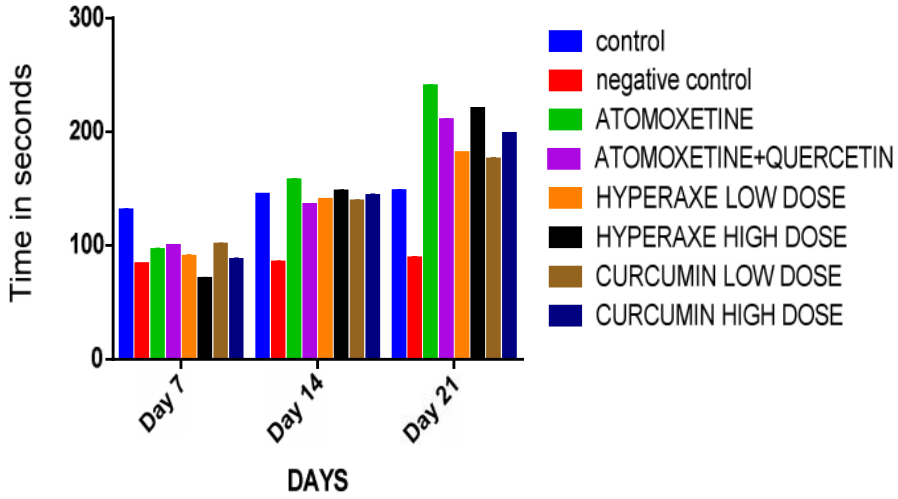


Table no: 11 Effect of *Hyperaxe, Curcumin, Quercetin* in Marble burying behavior:

S.no	Groups	Marbles Buried		
		Day 7	Day 14	Day 21
1	Control	12.6±0.1	11.3±0.02	12.6±0.1
2	Negative control	19.3±0.12	18.8±0.03	18.8±0.1
3	ATOMOXETINE	7±0.01	4.6±0.3	3.5±0.01
4	ATOMOXETINE+QUERCETIN	6±0.01 a**b**	4.5±0.2 a**b**	2.3±0.03 a**b**
5	HYPERAXE LOW DOSE	6.3±0.01 a**b**	5.3±0.01 a**b**	3.3±0.1 a**b**
6	HYPERAXE HIGH DOSE	9.3±0.02 a**b**	5.5±0.1 a**b**	1.6±0.04 a**b***
7	CURCUMIN LOW DOSE	8.1±0.01 a**b**	5.6±0.3 a**b**	4.5±0.05 a**b**
8	CURCUMIN HIGH DOSE	9.5±0.02 a**b**	6.16±0.1 a**b**	3±0.04 a**b**

Values are represented in Mean ± SEM, n=6
Comparison: a- Group II vs Group IV,V,VI,VII,VIII b- Group III vs Group IV,V,VI,VII,VIII 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, ****p<0.0001

Graph no: 8 Effect of *Hyperaxe, Curcumin, Quercetin* in Marble burying behavior:

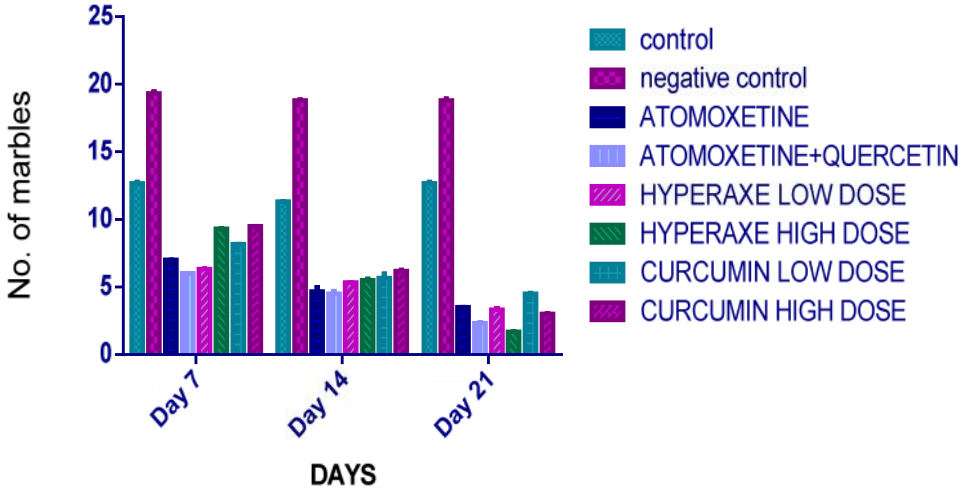


Table no: 12 Effect of *Hyperaxe, Curcumin, Quercetin* in Dopamine:

S.no	Groups	ng/mg wt tissue
1	Control	229±0.1
2	Negative control	58±0.1
3	Atomoxetine	213±0.12
4	Atomoxetine+quercetin	190±0.1 ^{a**b***}
5	Hyperaxe Low dose	180±0.13 ^{a**b**}
6	Hyperaxe High dose	205±0.01 ^{a**b***}
7	Curcumin Low dose	175±0.2 ^{a**b**}
8	Curcumin High dose	193±0.3 ^{a**b**}

Values are represented in Mean ± SEM, n=6
Comparison: a- Group II vs Group IV,V,VI,VII,VIII b- Group III vs Group IV,V,VI,VII,VIII 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, ****p<0.0001

Graph no: 9 Effect of *Hyperaxe, Curcumin, Quercetin* in Dopamine:

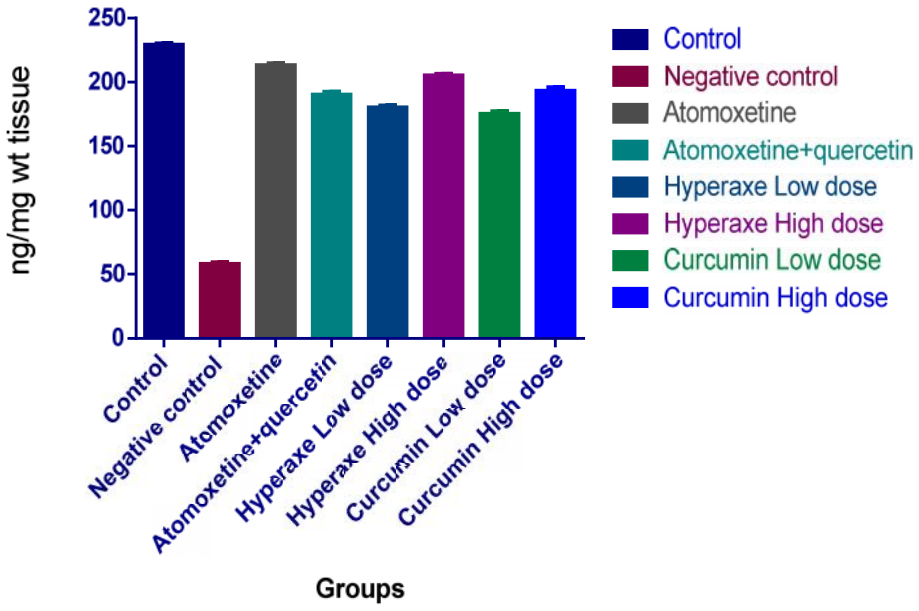


Table no: 13 Effect of *Hyperaxe, Curcumin, Quercetin* in Serotonin:

S.no	Groups	ng/mg wt tissue
1	Control	9±2.1
2	Negative control	22±3.2
3	Atomoxetine	13.4±2.1
4	Atomoxetine+quercetin	17.2±3.2 ^{a**b***}
5	Hyperaxe Low dose	19±3.01 ^{a**b**}
6	Hyperaxe High dose	14±2.43 ^{a**b***}
7	Curcumin Low dose	19.5±4.3 ^{a**b**}
8	Curcumin High dose	17.35±3.3 ^{a**b**}

Values are represented in Mean ± SEM, n=6
Comparison: a- Group II vs Group IV,V,VI,VII,VIII b- Group III vs Group IV,V,VI,VII,VIII 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, ****p<0.0001

Graph no: 10 Effect of *Hyperaxe, Curcumin, Quercetin* in Serotonin:

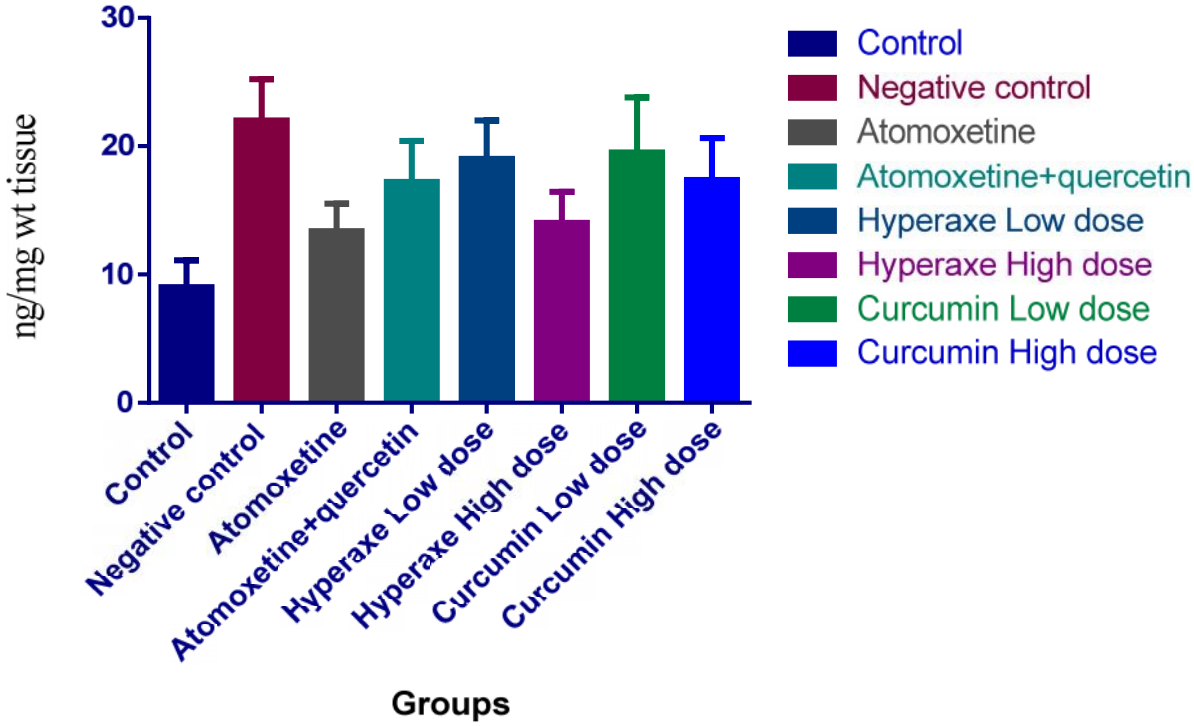


Table no: 14 Effect of *Hyperaxe, Curcumin, Quercetin* in Norepinephrine:

S.no	Groups	ng/mg wt tissue
1	Control	32.5±3.1
2	Negative control	34.45±4.2
3	Atomoxetine	33.55±4.1
4	Atomoxetine+quercetin	31.56±3.45 ^{a**b***}
5	Hyperaxe Low dose	34.56±3.01 ^{a**b**}
6	Hyperaxe High dose	33.67±2.43 ^{a**b***}
7	Curcumin Low dose	34.39±4.3 ^{a**b**}
8	Curcumin High dose	33.18±4.56 ^{a**b**}

Values are represented in Mean ± SEM, n=6
Comparison: a- Group II vs Group IV,V,VI,VII,VIII b- Group III vs Group IV,V,VI,VII,VIII 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, ****p<0.0001

Graph no: 11 Effect of *Hyperaxe, Curcumin, Quercetin* in Norepinephrine:

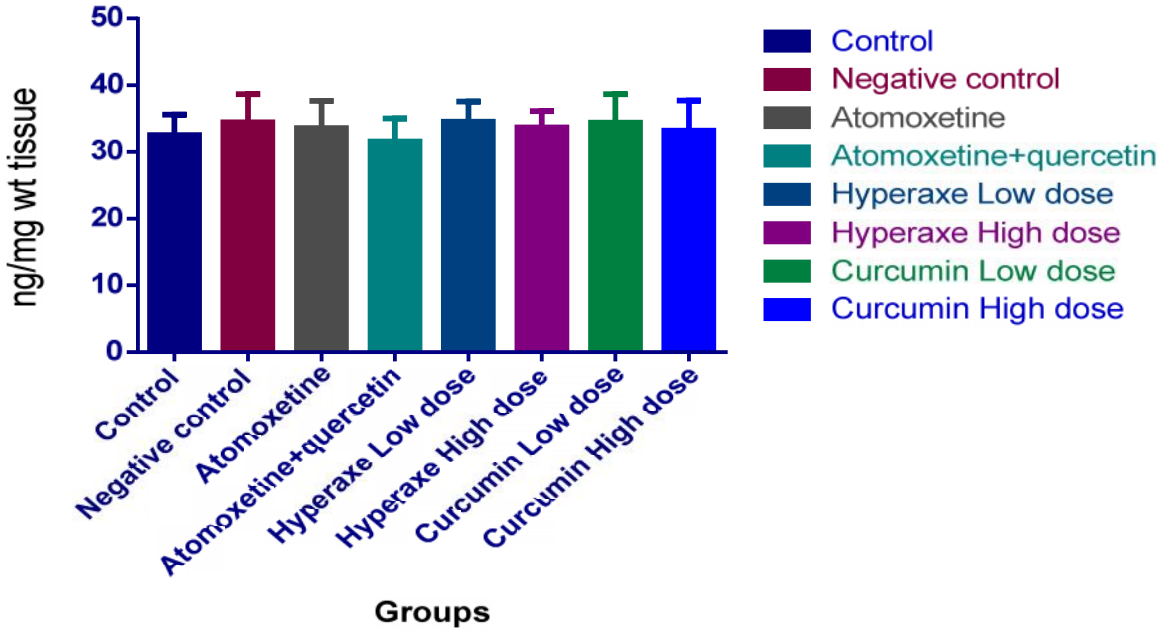


Table no: 15 Effect of *Hyperaxe, Curcumin, Quercetin* in Superoxide dismutase (SOD):

S.no	Groups	ng/mg Protein
1	Control	9.48±0.4
2	Negative control	4.51±0.25
3	Atomoxetine	9.02±0.41
4	Atomoxetine+quercetin	9.001±0.3 ^{a**b***}
5	Hyperaxe Low dose	5.01±0.21 ^{a**b**}
6	Hyperaxe High dose	8.01±0.14 ^{a**b***}
7	Curcumin Low dose	4.99±0.3 ^{a**b**}
8	Curcumin High dose	6.26±0.45 ^{a**b**}

Values are represented in Mean ± SEM, n=6
Comparison: a- Group II vs Group IV,V,VI,VII,VIII b- Group III vs Group IV,V,VI,VII,VIII 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, ****p<0.0001

Graph no: 12 Effect of *Hyperaxe, Curcumin, Quercetin* in Superoxide dismutase (SOD):

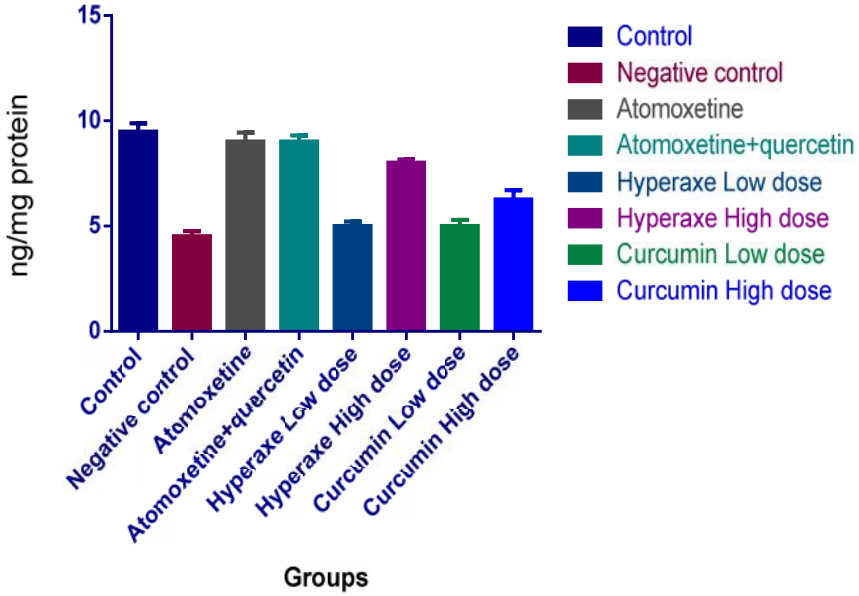


Table no: 16 Effect of *Hyperaxe, Curcumin, Quercetin* in Nitric Oxide (NO):

S.no	Groups	ng/mg Protein
1	Control	11.25±0.4
2	Negative control	5.3±0.25
3	Atomoxetine	8.3±0.41
4	Atomoxetine+quercetin	8.1±0.3 ^{a**b***}
5	Hyperaxe Low dose	6.5±0.21 ^{a**b**}
6	Hyperaxe High dose	9.2±0.14 ^{a**b***}
7	Curcumin Low dose	5.99±0.3 ^{a**b**}
8	Curcumin High dose	8.01±0.45 ^{a**b**}

Values are represented in Mean ± SEM, n=6
Comparison: a- Group II vs Group IV,V,VI,VII,VIII b- Group III vs Group IV,V,VI,VII,VIII 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, ****p<0.0001

Graph no: 13 Effect of *Hyperaxe, Curcumin, Quercetin* in Nitric Oxide (NO):

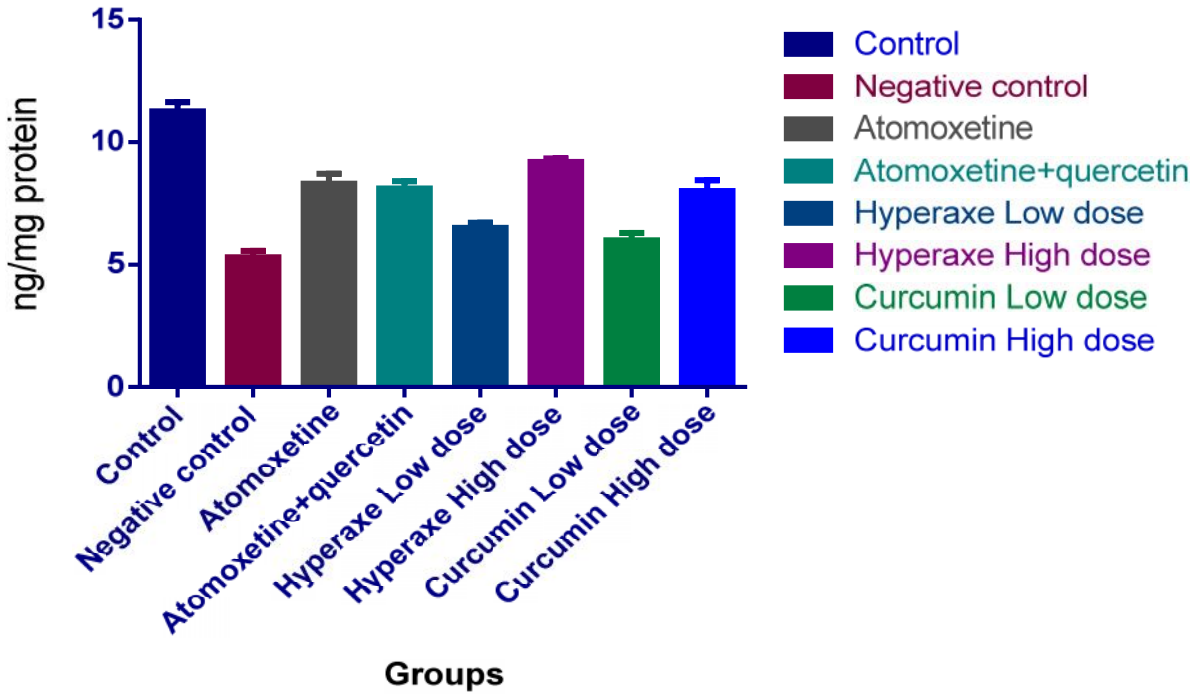


Table no: 17 Effect of *Hyperaxe* on SH-SY5Y Cell line:

S.no	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell Viability (%)
1	1000	Neat	0.15	23.80
2	500	1:1	0.22	34.92
3	250	1:2	0.27	42.85
4	125	1:4	0.33	**52.38**
5	62.5	1:8	0.38	60.31
6	31.2	1:16	0.44	69.84
7	15.6	1:32	0.49	77.77
8	7.8	1:64	0.56	88.88
9	Cell control	-	0.63	100

Graph no: 14:

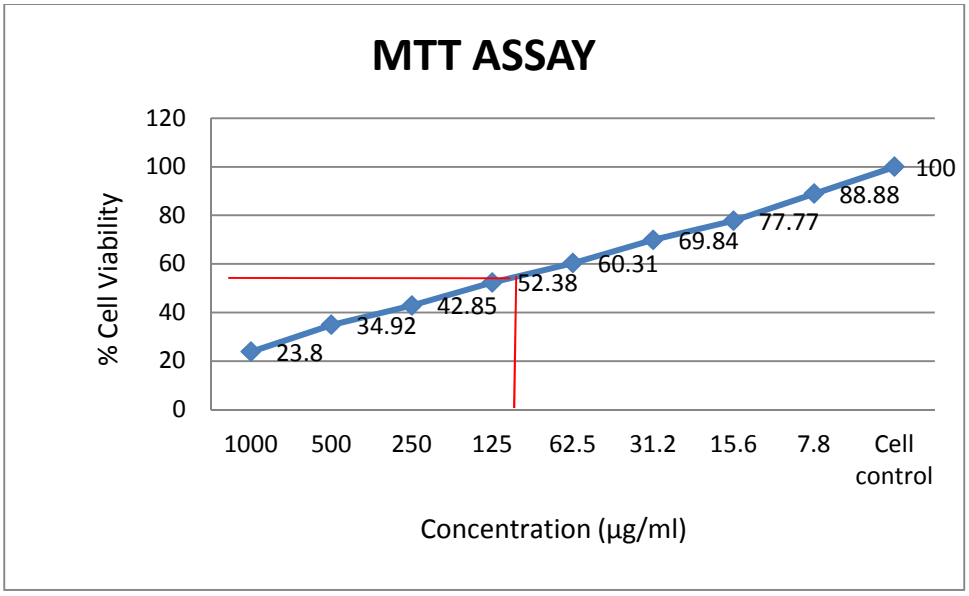
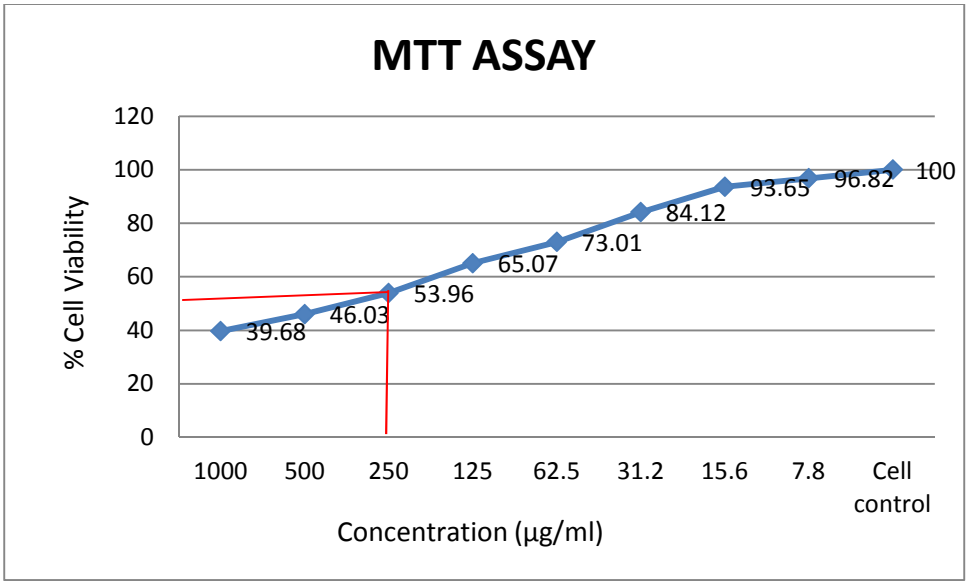


Table no: 18 Effect of *Curcumin* on SH-SY5Y *C*ell line:

S.no	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell Viability (%)
1	1000	Neat	0.25	39.68
2	500	1:1	0.29	46.03
3	250	1:2	0.34	**53.96**
4	125	1:4	0.41	65.07
5	62.5	1:8	0.46	73.01
6	31.2	1:16	0.53	84.12
7	15.6	1:32	0.59	93.65
8	7.8	1:64	0.61	96.82
9	Cell control	-	0.63	100

Graph no: 15:



23. RESULTS:

23.1 Preparation of Hyperaxe:

Successfully Hyperaxe is prepared by dispersing hydroalcoholic extracts of different plant materials in 0.5% Sodium carboxy methyl cellulose.

23.2 PRELIMINARY PHYTOCHEMICAL ANALYSIS

The preliminary phytochemical analysis on *Hyperaxe* revealed the presence of various phytoconstituents including alkaloids, carbohydrates, protein, steroids, phenols, tannins, flavonoids, gums, mucilage, etc. which are given in Table 2.

23.3 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 *Hyperaxe* was administered to the three Male SD rats and observed for fourteen 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 3.

23.4 ASSESSMENT OF IMPULSIVE BEHAVIOR & HYPERACTIVITY:

Reports included for day 21 only.

23.4.1 Effect of *Hyperaxe, Curcumin, Quercetin* in Actophotometer:

The Group II animals showed significant increase in locomotor activity when compared with Group I animals. Treatment with Atomoxetine+Quercetin, Hyperaxe (200 and 400mg/kg), Curcumin (200 and 400mg/kg) and the standard drug showed significant ($p<0.001$, $p<0.01$, $p<0.001$, $p<0.01$, $p<0.01$ for Group IV, V, VI, VII, VIII respectively) decrease in locomoter activity when compared with group II. Results are given in table 4 and plotted in graph 1.

23.4.2 Effect of *Hyperaxe, Curcumin, Quercetin* in Elevated Zero Maze:

The Group II animals showed decreased in percentage of alteration when compared with Group I animals. Treatment with Atomoxetine+Quercetin, Hyperaxe (200 and 400mg/kg), Curcumin (200 and 400mg/kg) and the standard drug showed significant ($p<0.001$, $p<0.01$, $p<0.001$, $p<0.01$, $p<0.01$ for Group IV, V, VI, VII, VIII respectively)

increase in time spent duration in open arm when compared with group II. Results are given in table 5 and plotted in graph 2.

23.5 ASSESSMENT OF COGNITIVE BEHAVIOR:

23.5.1 Effect of *Hyperaxe, Curcumin, Quercetin* in Morris water maze:

The escape latency of Group II animals was significantly similar when compared with Group I animals. Treatment with Atomoxetine+Quercetin, Hyperaxe (200 and 400mg/kg), Curcumin (200 and 400mg/kg) and the standard drug showed significant decrease ($p<0.001$, $p<0.01$, $p<0.001$, $p<0.01$, $p<0.01$ for Group IV, V, VI, VII, VIII respectively) in escape latency on to the hidden platform when compared with Group II. The decrease in escape latency indicates memory retention and non-spatial working memory. Results are given in table 6 and plotted in graph 3.

23.5.2 Effect of *Hyperaxe, Curcumin, Quercetin* in Pole Climbing test:

The escape latency of Group II animals was significantly similar when compared with Group I animals. Treatment with Atomoxetine+Quercetin, Hyperaxe (200 and 400mg/kg), Curcumin (200 and 400mg/kg) and the standard drug showed significant decrease ($p<0.001$, $p<0.01$, $p<0.001$, $p<0.01$, $p<0.01$ for Group IV, V, VI, VII, VIII respectively) in escape latency on to the hidden platform when compared with Group II. The decrease in escape latency indicates memory retention and non-spatial working memory. Results are given in table 7 and plotted in graph 4.

23.6 ASSESSMENT OF SKELETAL MUSCLE ACTIVITY:

23.6.1 Effect of *Hyperaxe, Curcumin, Quercetin* in Rod walking test:

The transfer latency of Group II animals was significantly increased when compared with Group I animals. Treatment with Atomoxetine+Quercetin, Hyperaxe (200 and 400mg/kg), Curcumin (200 and 400mg/kg) and the standard drug showed significant decrease ($p<0.001$, $p<0.01$, $p<0.001$, $p<0.01$, $p<0.001$ for Group IV, V, VI, VII, VIII respectively) in transfer latency on to the horizontal platform when compared with Group II. The decrease in transfer latency indicates memory retention and non-spatial working memory. Results are given in table 8 and plotted in graph 5.

23.6.2 Effect of *Hyperaxe, Curcumin, Quercetin* in Rota rod:

The fall off time of Group II animals was remarkably increased when compared with Group I animals. Treatment with Atomoxetine+Quercetin, Hyperaxe (200 and 400mg/kg), Curcumin (200 and 400mg/kg) and the standard drug showed significant decrease ($p<0.001$, $p<0.01$, $p<0.001$, $p<0.01$, $p<0.01$ for Group IV, V, VI, VII, VIII respectively) in fall off time when compared with Group II animals. Results are given in table 9 and plotted in graph 6.

23.7 ASSESSMENT OF OTHER PARAMETERS:

23.7.1 Effect of *Hyperaxe, Curcumin, Quercetin* in Dark-Light Compartment:

The percentage of alteration was significantly decreased in Group II when compared with Group I animals significantly. Treatment with Atomoxetine+Quercetin, Hyperaxe (200 and 400mg/kg), Curcumin (200 and 400mg/kg) and the standard drug showed significant increase ($p<0.001$, $p<0.01$, $p<0.001$, $p<0.01$, $p<0.01$ for Group IV, V, VI, VII, VIII respectively) in time spent duration in Light compartment when compared with group II. Results are given in table 10 and plotted in graph 7.

23.7.2 Effect of *Hyperaxe, Curcumin, Quercetin* in Marble burying behavior:

The no of marbles buried by Group II was more in number when compared with Group I animals. Treatment with Atomoxetine+Quercetin, Hyperaxe (200 and 400mg/kg), Curcumin (200 and 400mg/kg) and the standard drug showed ($p<0.001$, $p<0.01$, $p<0.001$, $p<0.01$, $p<0.01$ for Group IV, V, VI, VII, VIII respectively) that the marbles buried were less in number when compared with Group II. Results are given in table 11 and plotted in graph 8.

23.8 NEUROTRANSMITTERS ESTIMATION:

23.8.1 Effect of *Hyperaxe, Curcumin, Quercetin* in Dopamine:

The brain dopamine level in Group II animals was significantly decreased when compared with Group I animals. Treatment with Atomoxetine+Quercetin, Hyperaxe (200 and 400mg/kg), Curcumin (200 and 400mg/kg) and the standard drug showed significant ($p<0.001$, $p<0.01$, $p<0.001$, $p<0.01$, $p<0.01$ for Group IV, V, VI, VII, VIII respectively) increase in dopamine levels when compared with Group II. Results are given in table 12 and plotted in graph 9.

23.8.2 Effect of *Hyperaxe, Curcumin, Quercetin* in Serotonin:

The brain serotonin level in Group II animals was significantly increased when compared with Group I animals. Treatment with Atomoxetine+Quercetin, Hyperaxe (200 and 400mg/kg), Curcumin (200 and 400mg/kg) and the standard drug showed significant ($p<0.001$, $p<0.01$, $p<0.001$, $p<0.01$, $p<0.01$ for Group IV, V, VI, VII, VIII respectively) decrease in serotonin levels when compared with Group II. Results are given in table 13 and plotted in graph 10.

23.8.3 Effect of *Hyperaxe, Curcumin, Quercetin* in Norepinephrine:

The brain norepinephrine level in Group II animals was similar when compared with Group I animals. Treatment with Atomoxetine+Quercetin, Hyperaxe (200 and 400mg/kg), Curcumin (200 and 400mg/kg) and the standard drug showed no effect ($p<0.001$, $p<0.01$, $p<0.001$, $p<0.01$, $p<0.01$ for Group IV, V, VI, VII, VIII respectively) when compared with Group II. Results are given in table 14 and plotted in graph 11.

23.9 ESTIMATION OF ANTIOXIDANT ENZYME

23.9.1 Effect of *Hyperaxe, Curcumin, Quercetin* in Superoxide dismutase (SOD):

The SOD in the brain of Group II animals were decreased significantly ($p<0.001$) when compared with Group I animals. Treatment with Atomoxetine+Quercetin, Hyperaxe (200 and 400mg/kg), Curcumin (200 and 400mg/kg) and the standard drug showed significant increase ($p<0.001$, $p<0.01$, $p<0.001$, $p<0.01$, $p<0.01$ for Group IV, V, VI, VII, VIII respectively) in SOD levels on comparison with Group II. Results are given in table 15 and plotted in graph 12.

23.9.2 Effect of *Hyperaxe, Curcumin, Quercetin* in Nitric Oxide (NO):

The NO in the brain of Group II animals were decreased significantly ($p<0.001$) when compared with Group I animals. Treatment with Atomoxetine+Quercetin, Hyperaxe (200 and 400mg/kg), Curcumin (200 and 400mg/kg) and the standard drug showed significant increase ($p<0.001$, $p<0.01$, $p<0.001$, $p<0.01$, $p<0.01$ for Group IV, V, VI, VII, VIII respectively) in NO levels on comparison with Group II. Results are given in table 16 and plotted in graph 13.

23.10 IN VITRO CELL LINE:

23.10.1 Effect of *Hyperaxe* on SH-SY5Y *C*ell line:

The effect of *Hyperaxe* on SH-SY5Y cell line showed **52.38% cell viability** at 125µg/ml concentration at 570 nm. IC_{50} was plotted graphically. Results are given in table 17 and plotted in graph 14.

23.10.2 Effect of *Curcumin* on SH-SY5Y *C*ell line:

The effect of *Curcumin* on SH-SY5Y cell line showed **53.96% cell viability** at 250µg/ml concentration at 570 nm. IC_{50} was plotted graphically. Results are given in table 18 and plotted in graph 15.

23.11 SH-SY5Y cell line:

23.11.1 Cell Viability assay for the *Hyperaxe* on SH-SY5Y Cell line:

Normal SH-SY5Y Cell line

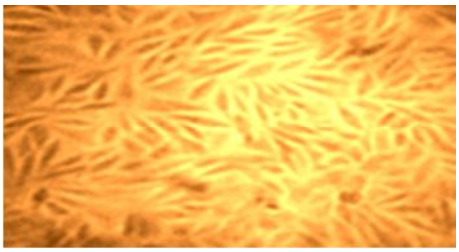


Fig no: 34

Fig no: 35 Toxicity-1000µg/ml

Fig no: 36 Toxicity-250µg/ml

Fig no: 37 Toxicity-125µg/ml

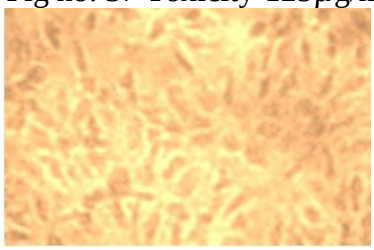
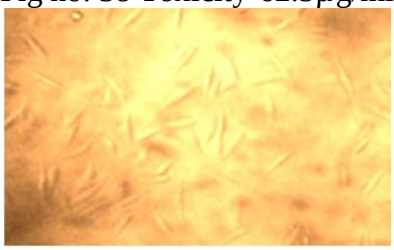


Fig no: 38 Toxicity-62.5µg/ml



23.11.2 Cell Viability assay for the *Curcumin* on SH-SY5Y Cell line:

Normal SH-SY5Y Cell line

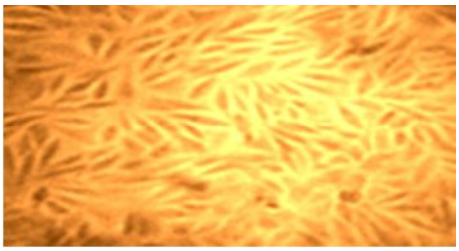


Fig no: 39

Fig no: 40 Toxicity-1000µg/ml

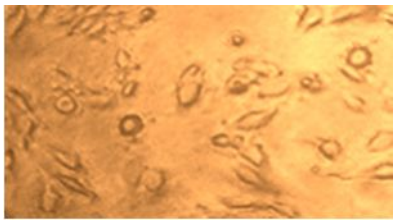


Fig no: 41 Toxicity-500µg/ml

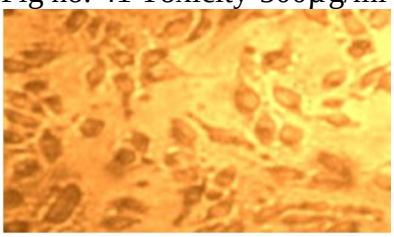


Fig no: 42 Toxicity-250µg/ml

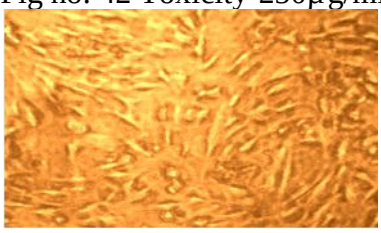


Fig no: 43 Toxicity-125µg/ml

23.12 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, VI, VII, and VIII) exhibited improved neuronal configuration than Group II. Group IV, V, VI, VII, and VIII 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

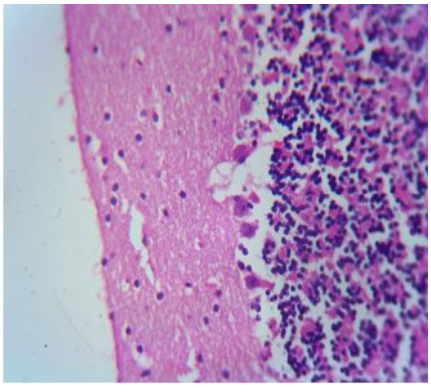


Fig no: 44 Control

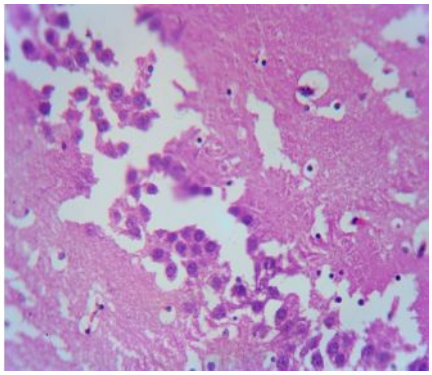


Fig no: 45 Negative Control

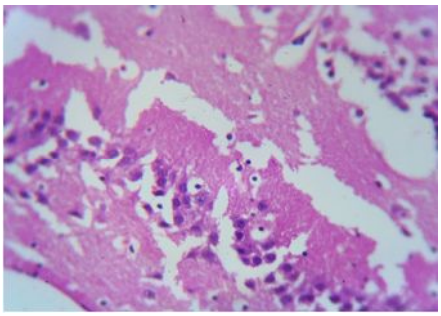


Fig no: 46 Atomoxetine

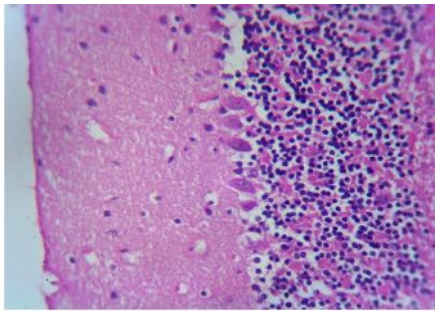


Fig no: 47 Atomoxetine+Quercetin

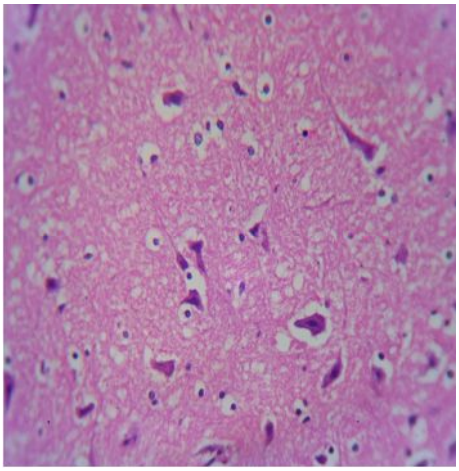


Fig no: 48 Hyperaxe low dose dose

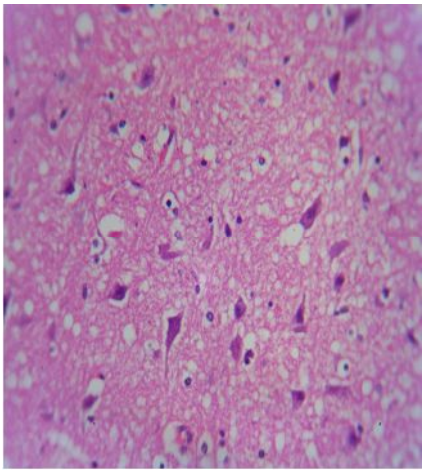


Fig no: 49 Curcumin low

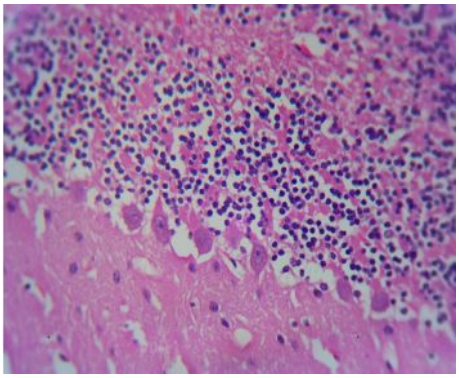


Fig no: 50 Hyperaxe high dose

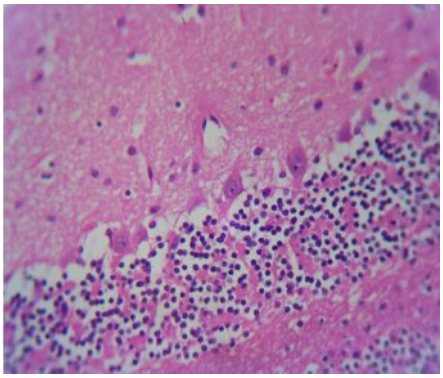


Fig no: 51 Curcumin high dose

24. DISCUSSION

ADHD is now the most common disorder in children and teens. The incidence of ADHD increases with age. Impairment of attention, impulsivity and hyperactivity is the prime and first clinical feature. When the condition progress, additional cognitive abilities are impaired as the ability to calculate and use common objects and tools. Although some studies and people with ADHD suggest that they have high learning capacity. Stimulants and Non-stimulants are the only agents approved by the Food and Drug Administration (FDA) for the treatment of ADHD. All other agents prescribed for the treatment of ADHD are used on an off- label basis.

Due to severe side effects of Drugs available to treat ADHD it becomes complicated to treat the symptoms. The patients may feel insomnia or may feel sedative with these stimulants and non-stimulants. Not all the patients with ADHD become comfortable with the prescribed drugs. Hence alternative treatments are suggested to decrease the symptoms of ADHD. Because the trio symptoms inattentive, impulsivity, hyperactivity cannot be cured with one drug. So herbal therapy like Ayurveda, Siddha and other plant based medicines are suggested to provide symptomatic relief to these patients.

Based upon literature review many plant based medicines are used to treat sypmtoms of ADHD. In literature review it has also been suggested that combination of plant extracts are greatly useful in symptomatic treatment of ADHD.

As per previous studies CURCUMIN and the combination of extracts in HYPERAXE has excellent antioxidant property, hence it is believed to have actions on CNS disorders and neurodevelopmental disorders.

The present study has revealed the ameliorative effect of HYPERAXE and CURCUMIN on 6-OHDA HBr induced Attention deficit hyperactivity disorder in SD neonates. 6-OHDA induced impairment of memory was assessed by using various behavioral parameters like Pole climbing apparatus and Morris water maze test. It was found that

treatment with HYPERAXE and CURCUMIN protect cognitive deficits in 6-OHDA HBr induced ADHD.

Spatial learning in the open field habituation was approached to access learning and memory. The decrease in response to a normal environment after repeated exposures to the familiar environment is referred to spatial habitual learning. Recurrent exposure produces a decrease in the exploratory initiatives, which is implicative of memory pertaining to a specific feature of that environment. Exploratory activities may be reduced on subsequent contact with closed field. In the result of this study reduced by the group of animals treated with HYPERAXE and CURCUMIN indicated increased spatial habitual learning and sleep deprivation decreased spatial habitual learning.

Impulsive and hyperactive behavior was assessed using Actimeter and Elevated zero maze based on locomotion in closed field and novel maze environment and treatment with HYPERAXE & CURCUMIN reported that the reduction in the locomoter activity and increased time spent duration in open environment when compared to 6-OHDA HBr animals which exhibited high locomoter count and time spent duration in closed field.

Anxiolytic behavior which has some linkage in ADHD was assessed by dark-light compartment and marble burying behavior. 6-OHDA HBr induced ADHD animals showed high anxiety behavior when compared with control groups and the deficit animals were treated with HYPERAXE & CURCUMIN which showed potent reduction in the anxiolytic behavior. It was concluded by that the deficit animals buried more no of marbles when compared with HYPERAXE & CURCUMIN group and also deficit animals spend more time in dark compartment when compared with treatment group.

Skeletal muscle activity was assessed by rod walking test and rota rod apparatus. 6-OHDA HBr induced animals showed high fall off time in rota rod apparatus and delayed rod walking time. Treatment with HYPERAXE & CURCUMIN in deficit groups greatly reduced fall off time and also the time taken for the animals to transfer from one to another in rod

walking was also reduced. This indicated that HYPERAXE & CURCUMIN has muscle relaxant property.

Morris water maze task represents more specific for spatial memory. The essential feature of this technique is that rats are placed into large circular pool of water and can escape into a hidden platform. Thus, the platform offers no local cues to guide escape behavior and the rat can escape from swimming by climbing on to the platform apparently learns the spatial location of the platform any starting position at the circumference of the pool. The only spatial cues are those outside water tank are primarily visual cues. Thus, the versatility of the task makes it a widely acceptable experimental model for the assessment of cognitive tests. Typically, 6-OHDA HBr induced animals exhibited an increase time for escape latency indicating loss of visual cues to escape to the platform. Such a diminished cognition was reversed by the administration of the HYPERAXE & CURCUMIN at the specified dosage levels and exhibited escape latency (EL), indicating the well-developed spatial memory inspite of 6-OHDA HBr induced ADHD.

Both adrenergic and dopaminergic receptors are involved in attention deficit hyperactivity disorder and several studies have suggested their roles in inattention, hyperactivity and impulsivity. Marked dopiminergic deficit is a hallmark of the pathogenesis of ADHD and various drugs including stimulants and non-stimulants have designed to target this dopaminergic receptor. There was a significant reduction in the level of dopamine in the animals and it was treated with HYPERAXE & CURCUMIN and increased dopamine reduced the hyperactivity and improved impulsivity and cognitive behavior which was impaired by 6-OHDA HBr induction.

Dopamine is the critical neurotransmitter modulating long term hyperactivity along with this serotonin was also involved.

The formation of new memories is thought to require the hippocampus and adjacent medial temporal lobe, but the final storage of memories is widely distributed by neocortical network. Lesion studies have suggested that there is a wide distribution of neocortical memory traces encoded in the strength of synaptic connections among neurons across large areas of the

neocortex. Although, α_2A and D receptors play a major role in learning and memory, serotogenic, hyperactive and impulsive effects have also been detected and on the region implicated in memory storage are richly innervated by the dopaminergic system.

It has been suggested that antioxidant might contribute to the prevention of ADHD. The superoxide dismutase (SOD) constitutes a mutually supportive team of defense against reactive oxygen species. The most remarkable effect of HYPERAXE & CURCUMIN is increased activity of SOD in brain. Treatment with HYPERAXE & CURCUMIN preserved the reduced SOD to that of the normal control.

Usually increased level of nitric oxide causes oxidative stress. The HYPERAXE & CURCUMIN has better action on nitric oxide, since the levels of nitric oxide is reduced in the treatment groups.

.

25. CONCLUSION

The developed method using pretreatment with imipramine instead of desipramine showed good result which was comparable with the standard method available in literature review. So, the Mechanism of action of desipramine is to protect noradrenergic neuron which was used as a standard and pretreatment with imipramine produced similar levels. Hence I conclude that desipramine which is difficult to procure in many countries instead of that imipramine can be used.

The combination of Atomoxetine and Quercetin significantly benefited in impulsive, cognitive behavior and stabilized the skeletal muscle activity.

The selected dose 200mg/kg and 400mg/kg of *Hyperaxe* showed significant action in impulsive, cognitive behavior and skeletal muscle activity but higher dose 400mg/kg showed better action than lower dose 200mg/kg.

The selected dose 200mg/kg and 400mg/kg of *Curcumin* showed significant action in impulsive, cognitive behavior and skeletal muscle activity but higher dose 400mg/kg showed better action than lower dose 200mg/kg.

The present study relieved the action of Atomoxetine+Quercetin, *Hyperaxe and Curcumin* on 6-OHDA HBr induced Attention deficit hyperactivity disorder in Sprague dawley neonate model. From the results it can be concluded that Atomoxetine+Quercetin, *Hyperaxe and Curcumin* has remarkable effect in memory enhancement and oxidative stress. Further studies are required for the identification of molecular level action and individual phytoconstitutent that may responsible for CNS action.

REFERENCES:

1. Ravishankar B and Shukla VJ Indian systems of medicine: A Brief profile, Afr. J. Trad. 2007; 4 (3): 319 – 337.
2. Aggarwal BB, sundaram C, Malani N, Ichikawa H. Curcumin: the Indian solid gold. Adv.Exp.Med.Biol.2007; 595: pg no 1-75
3. Herbal medicine introduction .http.//www.holisticonline.com/herbal-med /herb-intro.html
4. Kokate C.K.. Purothi. A.P. Gokhale S.B., Importance of herbal medicine.Pharmacognosy 2011; 1(6):pg 1-3
5. Farnsworth, N. R., Soejarto, D.D. 1991. Global importance of medicinal plants. In: Akerele, O, Heywood, V. & Synge, H (eds). Conservation of Medicinal Plants. Cambridge, UK; Cambridge University Press.
6. Kala Chandra Prakash, Sajwan B S "Revitalizing Indian systems of herbal medicine by the National Medicinal Plants Board through institutional networking and capacity building". Current Science 2007; 93 (6): 797–806
7. Mishra L.C. Ayurveda introduction for pharmacists. The Pharmaceutical journal 2006; 276:108-110
8. Kostic, V, Przedborski, S, Flaster, E., Steric, N.Early development of levodopa induced dyskinesia and response fluctuation in young onset Parkinson disease. Neurology 1991; 41: 202-205
9. Marcel Dekker, New York, U.S.A and Houghton, P.J., Howes M.J. Natural products and derivatives affecting neurotransmission relevant to Alzheimer's and Parkinson disease. Neurosignals 2005; 14: 6-22
10. Reynolds, Cecil R.; Goldstein, Sam (1999). Handbook of neuro-developmental and genetic disorders in children. New York: The Guilford Press. pp. 3–8. ISBN 1-57230-448-0
11. Murray RM, Lewis SW (September 1987). "Is schizophrenia a neuro-developmental disorder". Br Med J (Clin Res Ed) 295 (6600): 681–2. doi:10.1136/bmj.295.6600.681. PMC 1247717. PMID 3117295.

12. Pletikos, Mihovil; Sousa, Andre MM; et al. (22 January 2014). "Temporal Specification and Bilaterality of Human Neocortical Topographic Gene Expression". Neuron 81 (2): 321–332. doi:10.1016/j.neuron.2013.11.018.
13. Samaco RC, Hogart A, LaSalle JM (February 2005). "Epigenetic overlap in autism-spectrum neurodevelopmental disorders: MECP2 deficiency causes reduced expression of UBE3A and GABRB3". Hum. Mol. Genet. 14 (4): 483–92. doi:10.1093/hmg/ddi045.
14. Gogtay N et al. From the Cover: Dynamic mapping of human cortical development during childhood through early adulthood.Proceedings of the National Academy of Sciences, 2004, 101: 8174-8179
15. National Institute of Mental Health (NIMH) / University of California Los Angeles (UCLA). Time-lapse imaging tracks brain developing from ages 5 to 20. NIMH/UCLA
    a. Projectvisualizesmaturingbrain-availableat
    b. *www.loni.ucla.edu/~thompson/DEVEL/PR.html*
16. Dietrich K et al. Principles and practices of neurodevelopmental assessment in children: Lessons learned from the centers for children's environmental health and disease prevention research. Environ Health Perspect, 2005 113(10):1437-1446.
17. American Psychiatric Association (2013). Diagnostic and Statistical Manual of Mental Disorders (5th ed.). Arlington: American Psychiatric Publishing. pp. 59–65. ISBN 978-0-89042-555-8.
18. *https://en.wikipedia.org/wiki/Attention_deficit_hyperactivity_disorder*
19. *https://www.healthline.com/health/adhd/history#overview1*
20. *http://www.myadhd.com/causesofadhd.html*
21. *http://adhd-institute.com/burden-of-adhd/epidemiology/*
22. *http://www.indianjpsychiatry.org/article.asp?issn=0019-5545;year=2013;volume=55;issue=4;spage=338;epage=342;aulast=Venkata*
23. https://www.healthline.com/health/adhd/three-types-adhd
24. *https://www.healthline.com/health/adhd/facts-statistics-infographic*
25. Dey S. Automatic Detection of Brain Functional Disorder Using Imaging Data.

26. Stahl's essential Psychopharmacology 4th edition neuroscientific basis practical applications.
27. *http://www.webmd.com/add-adhd/adhd-stimulant-therapy#1*
28. *http://adhd-institute.com/disease-management/non-pharmacological-therapy/*
29. *https://www.healthline.com/health/adhd/herbal-remedies#overview1*
30. Russell VA, Sagvolden T, Johansen EB. Animal models of attention-deficit hyperactivity disorder. Behavioral and Brain functions. 2005 Jul 15;1(1):9.
31. Rodriguez-Pallares J, Parga JA, Munoz A, Rey P, Guerra MJ, Labandeira-Garcia JL. Mechanism of 6-hydroxydopamine neurotoxicity: the role of NADPH oxidase and microglial activation in 6-hydroxydopamine-induced degeneration of dopaminergic neurons. Journal of neurochemistry. 2007 Oct 1;103(1):145-56.
32. Kostrzewa RM, Brus R, Kalbfleisch JH, Perry KW, Fuller RW. Proposed animal model of attention deficit hyperactivity disorder. Brain research bulletin. 1994 Dec 31;34(2):161-7.
33. https://plants.usda.gov/java/
34. https*://www.flowersofindia.net/catalog/slides/Garlic.html*
35. *http://www.stuartxchange.com/Bawang.html*
36. *http://www.stuartxchange.org/Kondol.html*
37. *http://www.stuartxchange.org/bilogo.html*
38. *http://www.stuartxchange.com/TakipKohol.html*
39. https://en.wikipedia.org/wiki/Curcumin
40. https://en.wikipedia.org/wiki/Quercetin
41. C.K.Kokate, A.P.Purohit, S.B.Gokhale.Text book of Pharmacognosy47thedition. August 1, 2007.
42. *https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd_gl423.pdf*
43. Kamila et al., IJPSR, 2015; Vol. 6(1): 300-307. E-ISSN: 0975-8232; P-ISSN: 2320-5148
44. http*://www.ugobasile.com/products/catalogue/behaviour-mazes-tracking/item/74-40163-elevated-zero-maze.html*
45. Atomoxetine Increases Histamine Release and Improves Learning Deficits in an Animal Model of Attention-Deficit Hyperactivity Disorder: The Spontaneously

Hypertensive Rat by Li-Li Liu, Jie Yang, Ge-Fei Lei, Gui-Ju Wang, Yu-Wei Wang and Ruo-Peng Sun Department of Pediatrics, Qilu Hospital of Shandong University, Jinan, China

46. Roof RL, Stein DG. Gender differences in Morris water maze performance depend on task parameters. Physiol Behav 1999; 68 :81–6.
47. Cook L, Weidley E. Behavioral effects of some psychopharmacological agents. Ann N Y AcadSci 1957 Mar 14;66 (3): 740-52.
48. Soman I, Mengi SA, Kasture SB. Effect of leaves of Buteafrondosa on stress, anxiety, and cognition in rats. Pharmacol BiochemBehav 2004 Sep; 79 (1): 11-6
49. *http://med.stanford.edu/content/dam/sm/sbfnl/documents/resourcesharing/sop/rotarod.pdf*
50. *https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2504462/*
51. 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
52. 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.
53. 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.
54. Parul R, Saha P, Kundu SK. In vitro nitric oxide scavenging activity of methanol extracts of three Bangladeshi medicinal plants. The pharma innovation. 2013 Feb 1;1(12).
55. Schmidt AJ, Krieg JC, Clement HW, Gebhardt S, Schulz E, Heiser P. Impact of drugs approved for treating ADHD on the cell survival and energy metabolism: an in-vitro study in human neuronal and immune cells. Journal of Psychopharmacology. 2010 Dec;24(12):1829-33.
56. Life teck research centre Arumbakkam, Chennai.