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An investigation on anti-depressant activity of fresh fruit juice of Malus domestica in experimental animal models

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AN INVESTIGATION ON ANTI-DEPRESSANT ACTIVITY OF FRESH FRUIT JUICE OF *MALUS DOMESTICA* IN EXPERIMENTAL ANIMAL MODELS

BY

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Under The Guidance of

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I hereby declare that this dissertation entitled **"AN INVESTIAGTON ON ANTI-DEPRESSANT ACTIVITY OF FRESH FRUIT JUICE OF *MALUS DOMESTICA* IN EXPERIMENTAL ANIMAL MODELS"** *is a bonafide and genuine research work carried out by me under the guidance of* **DR. SATISH S Ph.D. Research Guide** *and* **Associate Professor, Department of Pharmacology, Srinivas College of Pharmacy, Mangalore, Karnataka.**

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DEDICATED TO MY PARENTS AND MY SISTER

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"Trust in the LORD with all your heart and lean not on your own understanding; in all your ways acknowledge Him and he shall direct your paths."

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Date: 9|4|2019

Place: Mangalore

AVRIN ROMITHA LOBO

LIST OF ABBREVIATIONS

^0C	: Degree centigrade
%	: Percentage
5-HT	: 5-hydroxytryptamine
5-HTP	: 5-hydroxytryptophan
a_2-AR	: α_2-Adrenoceptor
ACh	: Acetylcholine
ACTH	: Adrenocorticotrophic hormone
AOB	: Accessory olfactory bulb
AOT	: Acute oral toxicity
Am	: Amydaloid neclus
ANOVA	: Analysis of variance
ATP	: Adenosine triphosphate
BDNF	: Brain derived neurotrophic factor
BP	: Blood pressure
bpm	: Beats per minute
C	: Cerebellum
cAMP	: Cyclic adenosine monophosphate
CaMKII	: Calcium calmoduline dependent kinase II
cm	: Centimeter
CNS	: Central nervous system
CPCSEA	: Committee for the purpose of control and supervision on experiments of animals
CMC	: Carboxymethyl cellulose
CRH/CRF	: Cortocotropine releasing hormone/factor

CVS	: Cardiovascular system
COMT	: Catechol-O methyl transferase
DA	: Dopamine
DALY	: Disability-adjusted life years
DβH	: Dopamine-β-hydroxylase
DBP	: Diastolic blood pressure
DSM	: Diagnostic and statistical manual of mental disorders -fourth
DOPA	: 3,4 dihydroxyphenylalanine
E	: Epinephrine
EAAT	: Excitatory amino acid transporter
ECT	: Electroconvulsive therapy
EDTA	: Ethylenediamine tetra-acetate
EPM	: Elevated plus maze
FFJMD	: Fresh fruit juice of malus domestica
FMIR	: Functional magnetic resonance imaging
FST	: Forced swim test
g	: Gram
g/l	: Gram/litre
GABA	: Gamma-aminobutyric acid
GABA-A	: Gamma-aminobutyric acid-A
GABA-B	: Gamma-aminobutyric acid-B
GAD	: Glutamate decarboxylase
GPCR	: G protein–coupled receptors
Hcl	: Hydrochloric acid
HPA	: Hypothalamic pituitary adrenal axis

HPT axis	: hypothalamic-pitutary-thyroid (HPT) axis
Hip	: Hippocampus
Hyp	: Hypothalamus
i.p.	: Intraperitoneal
i.v.	: Intravenous
IFN	: Interferon
IL	: Interleukin
IAEC	: Institutional animal ethics committee
ICD	: International classification of diseases
IDO	: Indoleamine 2,3-dioxygenase
kg	: Kilogram
LC	: Locus coeruleus
LTP	: Long term potentiation
MD	: Malus domestica
mg/dl	: Milligram/ deciliter
mg/kg	: Milligram /kilogram
Min	: Minutes
MABP	: Mean arterial blood pressure
MAO	: Monoamine oxidase enzyme
MAO-A	: Monoamine oxidase enzyme-A
MAO-B	: Monoamine oxidase enzyme-B
MAO I	: Monoamine oxidase inhibitors inhibitors
mg/kg	: Milligram /kilogram
mm	: Millimeter
MDD	: Major depressive disorder

MFB	: Medial forebrain bundle
MOB	: Main olfactory bulb
NaOH	: Sodium hydroxide
nAChRs	: Neuronal nicotinic acetylcholine receptors
NE	: Norepinephrine
nm	: Nanometer
NRI	: Norepnephrine reuptake inhibitors
NSAID	: Nonsteroidal anti-inflammatory drugs
NMDA	: N-methyl-D-aspartate
NT	: Neurotransmitter
OBX	: Olfactory bulbectomy
OECD	: Organization for economic co-operation and development
OPT	: O-Phthalaldehyde
OFT	: Open field test
P	: Probability
p.o.	: Per oral
PFC	: Prefrontal cortex
PKA	: Protein kinase A
PVN	: Para ventricular nucleus
REM	: Rapid eye movement
RIMA	: Reversible inhibitor of monoamine oxidase-A
Rmp	: Rotations per minute
SBP	: Systolic blood pressure
sec	: Seconds
Sep	: Septum

S.D	: Standard deviation
S.E.M.	: Standard error mean
SN	: Substantia nigra
SSRI	: Selective serotonin reuptake inhibitors
SNRI	: Serotonin norepinephrine reuptake inhibitors
Str	: Corpus striatum
Tamb	: Ambient temperature
TCA	: Tricyclic antidepressant
Th	: Thalamus
TNF-α	: Tumor necrosis factor α
TST	: Tail suspension test
RF	: Brainstem reticular formation
UV	: Ultraviolet
VNO	: The vomeronasal organ
VTA	: Ventral tegmental area
WHO	: World health organization
w/v	: Weight/volume
YLDs	: Years lived with disability
µg/ml	: Microgram/milliliter
µl	: Microlitre

ABSTRACT

Objective:

To evaluate the anti-depressant effect of acute and chronic administration of fresh fruit juice of *Malus domestica* in experimental animal models.

Methods:

Anti-depressant activity of fresh fruit juice of *Malus domestica* was investigated in experimental animal models. Two doses 0.5ml and 1ml of FFJMD (oral route) was subjected for the evaluation as acute (1day) and chronic treatment (10days). Imipramine (10mg/kg oral) was used as standard in all the models of animals and parameters estimated includes estimation of biochemical parameter (mono amino oxidase).

Results:

Both the lower (0.5ml) and higher dose (1ml) of *Malus domestica fresh* fruit juice showed dose dependent significant decrease in depression. In acute and chronic forced swim test as well as acute tail suspension test, duration of immobility was significantly reduced in the FFJMD 1 ml and 0.5 ml treated group but the effectiveness was found more in FFJMD 1 ml. In hole board test there is increase in activity with FFJMD 0.5 ml and 1 ml treated groups and increase in biochemical parameter such as mono amino oxidase when compared with depressive control. The antidepressant activity of 1 ml was comparable to that of Imipramine 10 mg/kg.

Conclusion:

The present study suggests that fresh fruit juice of *Malus domestica* has antidepressant activity in both the doses but more beneficial effect was found in chronic administration at 1 ml. It would be advisable to encourage consumption of *Malus domestica* extract in patients with depression because of its nutritional and functional properties.

Keywords: Anti-depressant activity; Imipramine; *Malus domestica*

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Chapter 1

Introduction

INTRODUCTION

The human being is multifaceted, resembling a highly specialized and complicated machine. It operates as a single unit but is made up of a number of operational parts that work interdependently. Each part is related to a specific function that is necessary for the welfare of the human being. The component parts do not function independently, but rather in combination with all the others. Impaired function of one part, the consequences are likely to extend to other parts and may reduce the capability of the body to perform function normally. Integrated functioning of the body parts ensures the capability of the individual to live. The human being is, therefore, multifaceted in both its organization and function.[1]

Disease, decay and death have always co-existed with life, the study of diseases, disorder and their treatment must also have been contemporary with the dawn of the human intelligence. Abnormalities in any normal functioning of the body to maintain the homeostasis result in disease or disorder affecting individual‟s health.[2]

Among all the diseases and disorders, the most complex and disturbing all age groups are the psychological and neurological disorders. These disorders not only influence the individual‟s health and mental status but also its social, socioeconomic and family life.[3]

Everybody feels sad during his life for a temporary period that passes away with time. Depression disturbs the daily life and causes pain for himself and caring person. Most of the people never get proper treatment but somebody receive it properly. Depression can be treated with antidepressant drugs.[4]

Long-term changes in neural plasticity and neurogenesis could even alter the neural morphology and play an important role in the development of psychiatric disease and represent promising new remedial targets. These mechanisms open interesting perspectives for the design of new generations of psychotropic drugs. The modern techniques existing in neurobiology from the last decade, such as the different models of genetically modified mice and the leading neuroimaging approaches have provided definitive steps for the advancement of the understanding of these neurobiological disorders. Psychopharmacology as well as other medical disciplines still requires a great research effort for the achievement of new therapeutic strategies for the management of psychiatric diseases. The current advances in the facts of the neurobiological mechanisms underlying the physiopathology of these disorders are providing attractive indications for the design of these new strategies.[5]

The possible development of novel generations of psychoactive drugs selectively targeting key components of these emergent mechanisms can probably identify in a near future XIV more effective compounds and with fewer side effects than the drugs now available in the pharmaceutical market.[6]

Depression is a common mental disorder characterized by sadness, loss of interest or pleasure, feelings of low self-worth, disturbed sleep and poor concentration and is considered as an affective disorder characterized by change in mood, lack of interest in the surroundings, psychomotor retardation and melancholia.[7]

Major depression is characterized by the feelings of intense sadness and despair, mental slowing and loss of concentration, pessimistic worry, lack of pleasure, self-deprecation and variables agitation or hostility. Physical changes also occur, particularly in severe, vital or melancholic depression. Psychotic depression,

postpartum depression and seasonal effective disorder are also kinds of depression. Rather, it likely results from a combination of genetic, biochemical, environmental, psychological factors. However, depression can occur in people without family histories of depression as well.[8]

Depression is a burdensome psychiatric disorder that affects a person‟s mood, physical health, behaviour which is has been estimated to affect 450 million people according to the World Health Report. This amounts to 12.3% of the global burden of disease, and predicted to rise upto 15% to 20%. The disordernis also often associated with suicide and there are between 10 and 20 million suicide attempt every year. Depression is the most prevalent disorder and it is recognized to be symptomatically, psychologically and biologically heterogeneous.[9]

Depression shows a good response to pharmacological and behavioral treatments, individually or in combination. Among the various pharmacological agents selective serotonin reuptake inhibitors (SSRIs) and tricyclic antidepressants (TCAs) are most commonly used but have a lot of distressing adverse effects as they are often used for very long period of time.

Moreover, most of the patients respond to a single drug (most commonly a SSRI or a TCA) but only about 30% achieve remission (complete normalization of symptoms), thus, combination therapy of antidepressants with different mechanism of action or those having mixed effects on serotonin (5HT) and catecholamines i.e., norepinephrine (NE) and dopamine (DA) levels in brain are often required.

This also adds up to the adverse effects of individual drugs. Therefore, search for antidepressants with broader spectrum of action and a benign profile of adverse effect continues.[10]

As described in the Diagnostic and Statistical Manual of Mental Disorders, 5th

edition (DSM- V, 2013), the hallmark of major depressive disorder (MDD) is the occurrence of depressed mood (dysphoria) and loss of interest in activities that were rather pleasurable in the past (anhedonia) for a duration of at least two weeks. These symptoms must also be accompanied by at least four of the following manifestations such as changes in appetite or weight, sleep patterns, altered psychomotor activity, feelings of worthlessness or guilt, difficulty concentrating or making decisions and recurrent thoughts of death or suicidal ideation.

Even though there are plenty of drugs developed for the management of depression, one of the challenges in dealing with this disease is that a significant portion of the patients taking antidepressants fail to attain full remission. Some patients also develop treatment resistant depression in which the patients fail to respond to the available drugs or other therapeutic approaches.[11]

Depression is a heterogeneous disorder often mistaken for a single clinical mental illness. There are indeed diverse forms of depression that can either be mild or extremely severe conditions like psychotic depression in which the patients show symptoms such as hallucinations and delusions. Diagnosis of this disorder is complicated because of the co-occurrence of many other mental conditions such as anxiety disorders, including panic agoraphobia syndrome, severe phobias, generalized anxiety disorder, social anxiety disorder, post-traumatic stress disorder (PTSD) and obsessive-compulsive disorder (OCD).

This co-morbidity is commonly seen in elderly patients and is also associated with severity of somatic symptoms.[12]

The drugs of plant origin are gaining increasing popularity due to the side effects caused by synthetic medications and are being investigated for remedies of a number

of disorders including antidepressant activity. Ayurveda uses the plant, the plant products and active ingredients present in plants for treating various disorders. *Malus domestica* being one the most common edible fruit also possesses antidepressant activity.[13]

Number of plants being used for the treatment of depression. *"Malus domestica*" is one among them and also literature review revealed that fruits of *Malus domestica* possess antidepressant activity,[14] though there is paucity of scientific data for its antidepressant activity, hence the present study was selected to investigate antidepressant activity of *Malus domestica* in mice. On the basis of its use in Ayurveda for the treatment, it is hypothesized that "*Malus domestica*" may possess antidepressant activity which needs to be investigated. Hence the fruit is selected for the study.

Chapter 2

Need and Objectives of study

NEED AND OBJECTIVES

NEED FOR THE STUDY

Depression is the most most prevalent disorder and it is recognized to be symptomatically, physiologically and biologically heterogenous.[15] The drawbacks of using medications such as Seretonin- Noradrenaline Reuptake Inhibitors can cause side effects like feeling agitated, shaky or anxious, indigestion and stomach aches, diarrhea or constipation, loss of appetite, dizziness, insomnia or feeling very sleepy, headaches, less sex drive. The TCA‟s have side effects like dry mouth, slight blurring of vision, constipation, drowsiness, dizziness, weight gain, excessive sweating, heart rhythm problems such as palpitations or tachycardia.

The drugs of plant origin are gaining increasing popularity due to the side effects caused by the synthetic medications and are being investigated for remedies of a number of disorders including antidepressant activity. Ayurveda uses the plant, the plant products and active ingredients present in plants for treating various disorders.

The World Health Organization (WHO) has estimated that each year about 8, 77,000 people die from suicide. The WHO has further reported that suicide attempts are up to 20 times more frequent than completed suicides and that mental health disorders (particularly depression and substance abuse) are associated with more than 90% of all cases of suicide. In recent community surveys, 4–10% of the general population has experienced an episode of major depressive disorder within the past year.

Imipramine 10mg/kg orally one hour before the experiment is taken as standard.[16] The antidepressant activity will be assessed using forced swim test, hole

board test and tail suspension test models in Albino mice (20-25 grams).[17] Depression often starts at a young age. It affects women more often than men, and unemployed people are also at high risk. The risk of depression is greater in women than in men. Statistically, the risk of depression is generally 10% to 25% for women and 5% to 12% for men. However, people with chronic illnesses face a significantly higher risk -- between 25% and 33%.

The use of alternative therapies, such as acupuncture and medicinal herbs is on rise because of many side effects and toxicities associated with synthetic drugs have several limitations. Assurance of the safety, quality, and efficacy of medicinal plants and herbal products has now become a key issue in industrialized and in developing countries. Depressive conditions are treated using traditional medicine with considerable success. Although various modern drugs are used to treat these types of disorders their prolonged usage may cause severe side effects. So there is an urge to develop new therapeutic agents with minimum side effects.

From review of literature it is found that plants contain alkaloids, flavonoids, saponins, terpenoids, steroids, glycosides, tannins, and volatile oils etc., which are used to cure many diseases. One such plant is the species *Malus domestica*. It is used for the treatment of cardiovascular disease, asthma, obesity, pulmonary dysfunction and cancer. Apple products have been shown to prevent skin, mammary and colon carcinogenesis in animal models. However, literature survey reveals that no pharmacological work has been done on evaluation of its fruit juice for anti-depressant activity. Therefore, the present study is designed to evaluate the anti-depressant activity of the fresh fruit juice *of Malus domestica* in experimental animal models.

OBJECTIVES OF THE STUDY:

The main objective was to study was to evaluate the antidepressant activity of fresh fruit juice of *Malus domestica in* validated experimental animal models.

1. **Identification and collection**

   Identification, collection and authentication of fruits of *Malus domestica*.

2. **Extraction**

   Extraction of *Malus domestica* fresh fruit juice

3. **Preliminary Phytochemical Investigation**

   Screening of the extract to identify its chemical constituents.

4. To record & compare dose dependent anti-depressant activity in, vehicle treated, test formulation & Standard antidepressant drug in different animal model of depression

5. **Models of anti-depressant activity**
   a) Forced Swim Test
   b) Tail Suspension Test
   c) Hole Board Test

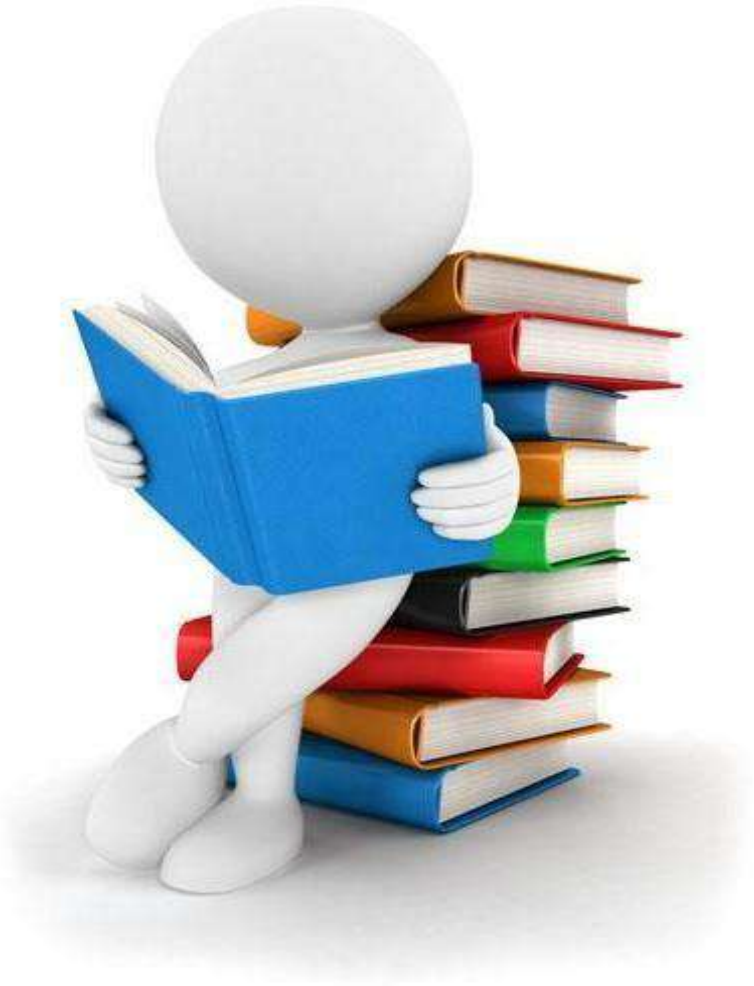
6. **Biochemical Estimation**

   - Measurement of Brain MAO-A activity

Criteria for the selection of the plant:

Number of plants being used for the treatment of depression. "*Malus domestica*" is one among them and also literature review revealed that fruits of *Malus domestica* possess antidepressant activity, though there is paucity of scientific data for its antidepressant activity, the present study was selected to investigate antidepressant activity of *Malus domestica* in mice. On the basis of its use in Ayurveda for the treatment, it is hypothesized that "*Malus domestica*" may possess antidepressant activity which needs to be investigated. Hence the fruit is selected for the present study.

Chapter 3



Review of literature

REVIEW OF LITERATURE

Central nervous system of a human being is very complicated as it contains more than 12 billion nerve cells. Along with endocrine system it control and coordinate various body functions. Depression has been described before a number of years. Hippocrates known as the father of western medicine initially used the term melancholia around 400 B.C.[18]

The 11th century, Avicenna, a Persian physician explained melancholia as a mood disorder.[19]The term depression also began to appear in the 19th century to indicate a state of sadness. Most of the major symptoms of depression observed today were recognized in ancient times.

Since the 1960s, on the basis of diagnostic and statistical manual by American Psychiatric Association 2000,[20] it is diagnosed as major depression. Depression is an extremely common psychiatric condition. It is the most common mood affective disorder which refers to a pathological change in mood state; depression varies from mild to severe depression accompanied by hallucinations and delusions.[21] It is recognized to be symptomatically, psychologically and biologically heterogenous.[22]

World Health Organization (WHO) lines depression as the fourth leading cause of disease worldwide with projection that by 2020, it will be second-ranked disease.[23] Symptoms of depression in human being include loss of concentration, hypersomnia, altered eating pattern, weight loss, over eating, disruption of normal circadian and ultradian rhythms, body temperature and alteration in many endocrine functions. Depressed patients usually act in response to antidepressants. Antidepressant drug acts by altering the monoamines metabolism and their receptors.[24]

3.1 Depression

Mental and behavioral disorders are commonly classified using the international classification of diseases-ICD-10 and an American psychiatric association has developed a precise system of diagnosis, based on the description of symptoms in the diagnostic and statistical manual of mental disorders.[25]

3.1.1 Diagnostic criteria for major depression

According to the diagnostic and statistical manual of mental disorders[26] an episode of major depression is diagnosed as a period of 2 weeks or longer in which a patient

presents with 5 or more of the symptoms listed in Table 1. These symptoms disrupt the normal social and/or occupational functioning of the patient. The presence of at least one of the first two symptoms in the list is a prerequisite for a diagnosis of major depression to be made.

Table 1: Diagnostic criteria for the major depression

Sl. No	Diagnostic criteria
1.	Depressed mood
2.	Diminished interest or pleasure in most activities
3.	Significant weight loss or gain, or decrease or increase in appetite
4.	Insomnia or hypersomnia
5.	Psychomotor agitation or retardation
6.	Fatigue or loss of energy
7.	Feelings of worthlessness, or excessive or inappropriate guilt
8.	Diminished ability to think or concentrate or indecisiveness
9.	Recurrent thoughts of death, suicidal ideation without a specific plan.

It is clear from the criteria in Table 1 that the depression is characterized as a heterogeneous syndrome consisting of numerous distinct symptoms.

3.1.2 Subtype of depression

Attempts have been made to establish subtypes of depression defined by certain sets of symptoms.[27] However, these subtypes are based solely on symptomatic differences and there is as yet no evidence that they reflect different underlying disease states.

Table 2: Examples of proposed subtypes of depression

Depression Subtype	Main Features
Melancholic (endogenous)	Severe symptoms; prominent neurovegetative
Depression	abnormalities.
Reactive (exogenous)	Moderate symptoms; apparently in response to
Depression	external factors.
Psychotic depression	Severe symptoms; associated with psychosis:
	e.g., believing depression is a punishment for
	past errors (a delusion) or hearing voices that
	depression is deserved (a hallucination).
Atypical depression	Associated with labile mood, hypersomnia,
	increased appetite, and weight gain.
Dysthymia	Milder symptoms, but with a more protracted
	course.

3.2 Epidemiology

As per the survey of WHO, depression is the most important cause of disability and become the foremost cause of disease and will be the second leading cause of disease in the year 2020. Alteration in mood occurs in the normal daily life of everyone. Women‟s are more susceptible to depression as compared to the men. Depression may occur at any ages, the average age of onset of depression is in the mid-20s. The peak level of depression in women occurs at the age of 35-45 years.[28] It is estimated that 3-4% of India‟s population suffers from major depressive disorders and 7-10% of the population suffers from minor depressive disorders. The study conducted in the Goa, India, reported the rate of depressive disorders was 46.5% in the adult.

Table 2. Represent the disability due to depression is more severe as compared to disability due to all forms of cancer and diabetes mellitus combined, as well as exceeding the disability due to strokes and hypertensive heart diseases.[29]

Table 3: Age-standardized DALYs per 100,000 population 2004 WHO figures for Goa, India

Disorder	Disability-adjusted life years
Neuropsychiatric disorders	3228
Depression	1401
CVS diseases	3521
Hypertensive heart disease	72
Cerebrovascular accidents	837
Cancer	984
Diabetes mellitus	305

The introduction of the American psychiatric association diagnostic and statistical manual (DSM-III in 1980 and DSM-IV in 1994) and the World Health Organization, international classification of diseases (ICD-10 in 1992) has resulted in the development of operational criteria for mental and behavioral disorders. This, in turn, has made it possible to perform large cross-sectional epidemiologic surveys to compare prevalence rates across various cultures and communities and between primary and secondary care settings.

Depression is prone to occur with every class of medication, includes antiarrhythmic, antihypertensive, β-adrenergic blockers and calcium channel blockers. The patient receiving glucocorticoids, antimicrobials, systemic analgesics, antiparkinsonian medications, anticholesterolemic agents, and anticonvulsants frequently show depression. Between 20 and 30% of cardiac patients, 40-50 % of patients with cancer of pancreas or oropharynx, 8-27 % patients with diabetes mellitus manifest depressive

disorder.[30] Depression is the most frequent mental disorder. More than 20% of the adult populations suffer from these conditions at some time during their life.[31] Both male and female prone to depression but the lifetime prevalence rate in women is 1.7-

2.7 times greater than men. The risk of depression is increased stepwise from early adolescence to their mid-50s.[32] Adults having age group of 25 - 44 susceptible for the highest rate of depression, leading to suicide attempt.[33]

The study conducted in southern India, Chennai among different age groups male and female, reported the prevalence of depression in females was significantly higher than men in all age groups.

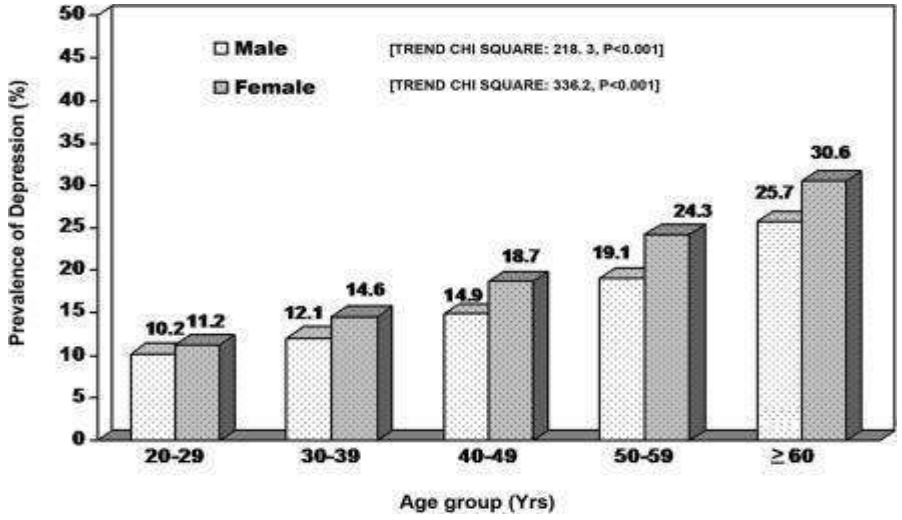


Figure 1: Lifetime prevalence of major depression in male and female.[34]

The study also reported that the individuals with aged 65 to 80 showed a greater lifetime prevalence of major depression.[35]

As stated earlier depression is associated with several factors, reported evidence suggest that heredity may be the one of the reason of depression. Women are more susceptible to depression than men influenced by genetic and environmental factors.

Approximately 8% -18% genetic and 39% and 61% environmental factor are responsible for the progress of depression.[36]

3.3 Etiology

Like most psychiatric disorder, the cause of affective disorder remains unknown in depression. Following factors which make the person susceptible to depression include, biochemical,endocrine, genetic, environmental and hormonal.[37]

3.3.1 Biochemical factor

Depression is associated with deficiency of neurotransmitter in a certain region of the brain includes dopamine, norepinephrine, and 5-hydroxytryptamine. Antidepressants increase the level of this neurotransmitter in the brain.[38]

3.3.2 Endocrine factor

Overactivity of HPA axis is associated with the affective disorder. Cushing‟s syndrome and hypothyroidism is connected with HPA and HPT axis dysfunction.[39]

3.3.3 Genetic factor

Serotonin transporter gene is a risk factor for depression. Many common disorders which are also influenced by genes include high blood pressure and diabetes**.** Cystic fibrosis and Huntington's disease may be caused due to the single defective gene.

The gene responsible for depression, 3p25-26 chromosome isolated by British research team appears to be present in many families with recurrent depression.[40]

3.3.4 Environmental factor

Environmental factors responsible for depression such as water, air, synthetic chemicals, food additives and food pollution, hormones, pesticides, drugs and industrial byproducts are bombarding our bodies at an extreme rate**.** Other sources include stress electrical pollution, natural disasters, noise pollution and other catastrophic environmental events. Some events include the death of a loved one, divorce, job loss, financial problems and disabling illness or injury sometimes called as social and relational causes of depression.[41]

3.3.5 Common medical disorders, psychiatric disorders, and drug therapy associated with depression

Disorders of mood, particularly depression, have been associated with several types of medication and a number of physical illnesses listed below in the Table.4

Table 4: Drug and physical illness implicated in disorders of mood.

Sl. no.	Medical disorders and diseases
1.	Endocrine diseases: - hypothyroidism, addison's disease, cushing's disease
2.	Deficiency state: - pernicious anemia, wernicke‟s encephalopathy
3.	Metabolic disorder
4.	Electrolyte imbalance, hypokalemia, hyponatremia
5.	Cardiovascular diseases
6.	Congestive heart failure, coronary artery disease, myocardial infarction
7.	Neurologic disorder
8.	Epilepsy, alzheimer's disease, huntington disease, multiple sclerosis, pain, parkinson‟s disease, malignant disease.
9.	Psychiatric disorder: -alcoholism, schizophrenia, anxiety disorder, eating disorder
10.	Drugs therapy
11.	Analgesics, antihypertensives, antipsychotic
12.	Antidepressants, anticonvulsants, antiparkinsonian agent
13.	Opiate withdrawal, benzodiazepine withdrawal
14.	Hormonal therapy - oral contraceptives, adrenocorticotropic hormone
15.	Others-Interferon

3.4 The Neuroanatomy of depression

Depression is associated with the many brain regions like the hippocampus, basal ganglia and amygdala and cortical brain regions.[42] Abnormalities in the structure and function of these regions in patient‟s exhibit distinct pathological changes in the brain and have been found to be associated with depression.

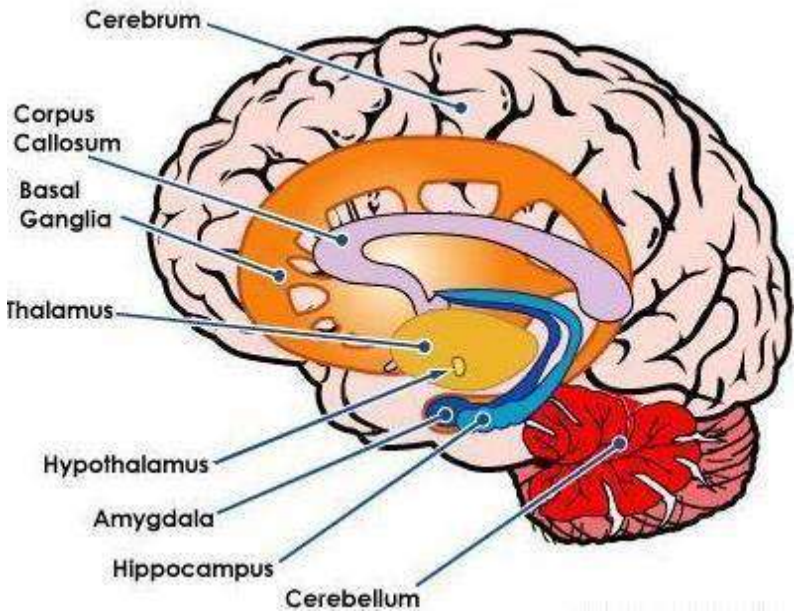


Figure 2: Parts of Brain

3.4.1 Amygdala

The amygdala part of the limbic system, are two almond shape masses of neurons on either side of thalamus linked to emotions like anger, sorrow, fear, pleasure and sexual arousal**.** A charged memory is responsible for activation of the amygdala.

Functional neuroimaging studies have supported the hypothesis that the amygdala is abnormally hyperactive in depressed patients.[43] Apart from the positron emission tomographic findings, several functional magnetic resonance imaging (fMRI) studies have demonstrated the hyperactivity of amygdala in depressed patients compared to controls.[44]

Amygdala regulates neuroendocrine response and cortical arousal as well as emotional learning and memory. It also stimulates the hypothalamus CRF-containing neurons to result in the release of corticotropin.[45] The rumination which has been found to be associated with amygdala activation may also play a role in bipolar

depression and anxiety.[46] Enlargement of the amygdala in first-episode depression may result from higher amygdala metabolism and blood flow. Additionally, recurrent depression episode of long duration result in amygdala shrinkage.[47]

3.4.2 Hippocampus

The hippocampus is one of the divisions of the limbic system and consists of two horns that curved back from the amygdala and has an important role in the translation of short-term memory to long-term memory recollection and responds to stress hormones in the blood.

Neuroimaging has been used to map actual changes in the brain structure of depressed patients related to their symptoms. Some imaging studies of brain structure have shown a reduction in hippocampal volume that reflects chronicity of depression.[48]

The dentate gyrus is a part of the hippocampus and/or hippocampal formation, where new neurons are produced throughout adult life. The physiological effects of stress slow the new neurons production in the dentate gyrus resulingt in loss of hippocampus volume that leads to increased neuronal cell death.[49] The pathophysiology of depression indicates impairment in negative feedback control of the HPA axis resulting in elevated corticosteroid level. Prolonged exposure to elevated levels of glucocorticoids result in hippocampal atrophy[50] and can induce neuronal apoptosis and possibly permanent damage.[51]

3.4.3 Prefrontal cortex

The prefrontal cortex is the component of the frontal lobe. It plays an important role in modulating the activity of limbic region and basal ganglia and has cortical and subcortical interconnections. It regulates emotion, volition, mood, memory, motivation and decision-making.[52] The prefrontal cortex (PFC) processes and modulates the physiological, neuroendocrine and behavioral responses (via the amygdala) and is also involved in fear and anxiety related conditional responses.[53] Neuroimaging study state that frontal cortex volume reduction ranges from 7%- 48% in the subgenual prefrontal cortex in major depression.[54] A number of recent studies have examined loss in frontal volume in major depressive disorder.[55]

3.4.4 Thalamus

The thalamus is involved in the transmission of sensory information and regulation of learning speech, behavioral reactions, movement, and thinking**.** The problem in thalamus leads to bipolar depression.[56]

3.4.5 Olfactory system

The components of the olfactory system include olfactory receptors, olfactory bulb, and nervous terminals. The olfactory receptors also called receptors for smell (olfaction) are chemoreceptors and are located in the olfactory epithelium. These are G-protein coupled receptors. The axons of olfactory receptors generate impulses carried by the olfactory nerves (1st cranial) to the olfactory bulbs in the temporal lobes of the forebrain.[57]

The 4% of the total brain mass comprise of the olfactory bulb in an adult rat.[58] Amputation of olfactory bulb shows the major impact on brain functions. It could result from disruption of local blood supply together with retrograde, anterograde and transneuronal degeneration.[59] It leads to different changes at the cellular level that result in synaptic number and function like synaptic sprouting and removal of nerve supply. The two chemosensory organs in the nasal cavity are called the olfactory epithelium and the vomeronasal organ (VNO).

The olfactory epithelium collects and sends information to the main olfactory bulb (MOB) and vomeronasal organ sends information to the accessory olfactory bulb (AOB. A third component and nervous terminals innervate both olfactory epithelium and the vomeronasal organ (VNO).

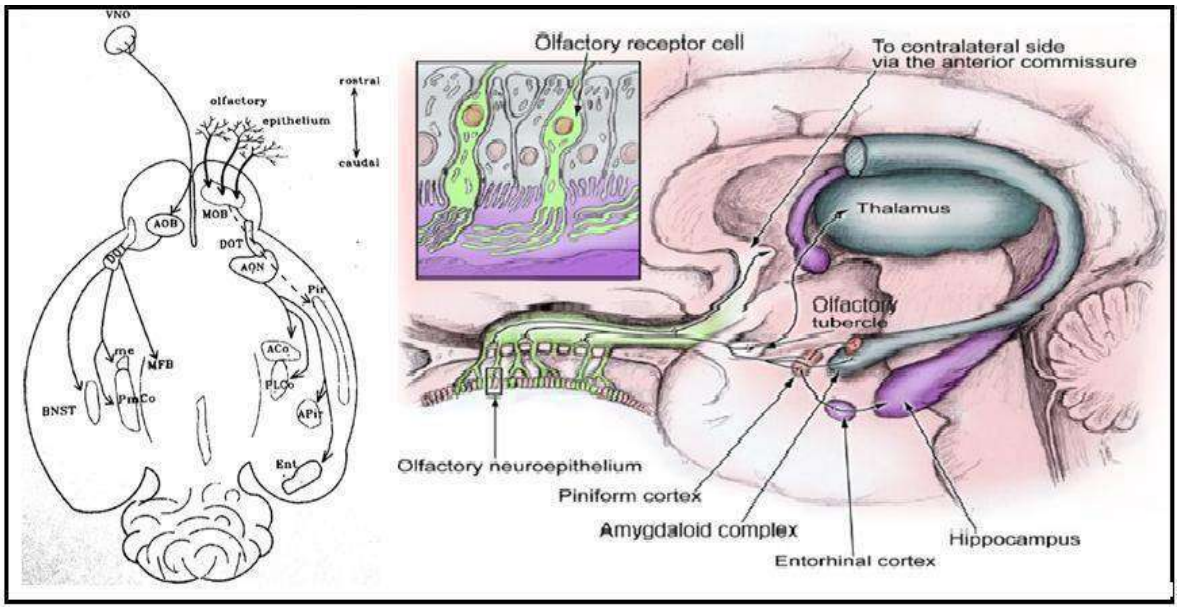


Figure 3: The connections of main and accessory olfactory bulbs.[60]

There is six well-defined region of the main olfactory bulb (MOB) divided into the cell of the external and inner layer respectively. The outermost layer is composed of cells such as glomeruli (discrete spheres of nerve tissue), tufted cells and mitral cells in olfactory bulb communicated with posterior pyriform cortex, amygdala and bed nucleus of stria terminal via neurotransmitters which are mainly dopaminergic and GABAergic.[61] The innermost layer is composed of tufted and mitral cells, acetylcholine, serotonin, noradrenaline, enkephalin and substance-P containing neurons.[62] The mitral cell and tufted cell form the main projection neuron and both send their axon into the olfactory tract.[63]

3.5 Neurotransmitters in depression.

3.5.1 Criteria for neurotransmitter

- It is synthesized and released from neurons.
- It is released from nerve terminals in a chemically or pharmacologically identifiable form.
- It reacts with postsynaptic receptors and brings about the same effects as are seen with stimulation of the presynaptic neuron.
- Its interaction with the postsynaptic receptor displays a specific pharmacology

- Its actions are terminated by active processes.[64]

3.5.2 Monoamines

The monoaminergic systems (e.g., serotonin, norepinephrine, dopamine) play an important role in the control of cognition, motor function, endocrine secretion, chronobiologic rhythms and appetite.[65] Most of the antidepressant drugs used in clinical practices act by increasing the concentration of monoamines in the synaptic cleft by neuronal reuptake inhibition or by blocking α2 auto and heteroreceptors.[66]

3.5.2.1 Synthesis, release and metabolism of monoamines.

Catecholamines consist of the benzene ring to which hydroxyl group i.e. catechol moiety attach and amine side chain. Pharmacologically the most important ones are

- **Noradrenaline** (**norepinephrine**)(NE), a transmitter released by sympathetic nerve terminals,
- **Adrenaline** (**epinephrine)**, a hormone secreted by the adrenal medulla,
- **Dopamine(DA)**, the metabolic precursor of noradrenaline and adrenaline also a transmitter / neuromodulator in the CNS and/or hormones in the periphery.

Catecholamines are formed in the brain, enterochromaffin cells, sympathetic nerves and sympathetic ganglia.[67] These three monoamines (epinephrine, norepinephrine, and dopamine) involve a common path in their synthesis. A precursor, L-tyrosine in the presence of tyrosine hydroxylase converted to 3, 4 dihydroxyphenylala- nine (DOPA) (rate limiting step). Enzyme tyrosine hydroxylase appears only in the catecholaminergic neuron. DOPA is then converted to dopamine and then into NE by enzyme dopa-decarboxylase and dopamine- β-hydroxylase respectively. Norepinephrine is converted to epinephrine in the adrenal medulla. Synaptic vesicles act as a storage house where norepinephrine is stored with adenosine triphosphate, chromogranin A and dopamine- β-hydroxylase.[68]

The indoleamine serotonin (5-HT) is a transmitter that is synthesized within the nerve ending from the amino acid tryptophan. In chromaffin cells and neuron conversion of tryptophan to 5-hydroxytryptophan take place in the presence of enzyme tryptophan hydroxylase.5-hydroxytryptophan is decarboxylated to serotonin by decarboxylase.[69]

The arrival of the action potential to nerve terminals opens the voltage - activated

calcium channels. Entry of calcium promotes vesicle fusion with the presynaptic membrane that leads to the release of monoamine into the synaptic cleft by exocytosis process.[70]

This release neurotransmitter acts on a specific receptor on presynaptic or postsynaptic membrane (Figure 4).

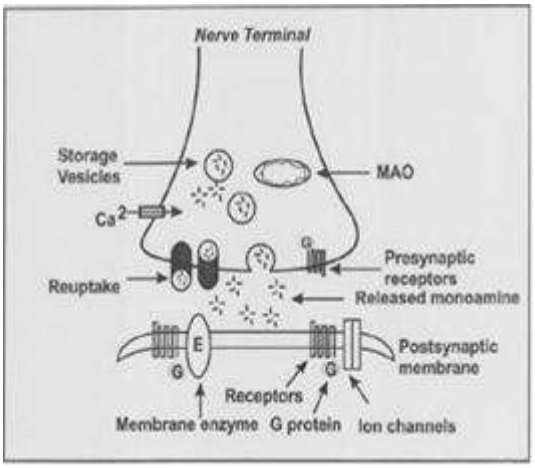


Figure 4: The monoamine neuron

The stimulation of postsynaptic receptors changes the postsynaptic membrane potential.

Coupling of the inotropic receptor with neurotransmitter causes changes in membrane potential and coupling of G protein metabotropic receptor with neurotransmitter result in biochemical changes viz. activation of intracellular second messenger system.[71]

The feedback mechanism maintains the concentration of the neurotransmitter in the synaptic cleft.[72]

The pre-synaptic regulatory receptors when present on the same neuron viz. α2 receptor present on noradrenergic neuron called autoreceptors, but when α2 present on another serotonergic neuron is called heteroreceptors.

The autoreceptors located in any part of the cell membrane such as cell body, dendrites, axon or axon terminals,[73] play an important role in balancing the amount of neurotransmitter. These are inhibitory in nature. These receptors regulate synthesis and secretion of neurotransmitter in vesicle and control the concentration of it in the synaptic cleft.

The released monoamine undergoes rapid metabolism by the enzyme monoamine oxidise(MAO) and catechol-O-methyl transferase (COMT). The monoamine oxidase exists in two different forms, MAO-A, and MAO-B.[74]

3.6 Pathways in the brain

3.6.1 Norepinephrine pathway in the CNS

The distributions of a noradrenergic and serotonergic neuron are similar in pons and medulla. The cell bodies of this neuron extensively send the axons to different parts of the brain and spinal cord (Fig. 5). The most prominent cluster is the locus coeruleus (LC) located in the pons. Other noradrenergic neurons lie close to the LC in the pons and medulla and widely project to the hypothalamus, hippocampus, prefrontal cortex and other parts of the forebrain as well as to the cerebellum and spinal cord.

The locus coeruleus is involved in the descending control of pain pathways. The regulation of the stressful environmental response correlates with the involvement of the norepinephrine system in the pathogenesis of depressive and anxiety disorders.[75]

A small cluster of epinephrine neurons which release epinephrine rather than norepinephrine lies more ventrally in the brain stem, projecting mainly to the pons, medulla and hypothalamus. Rather little is known about them but they are believed to be important in cardiovascular control.

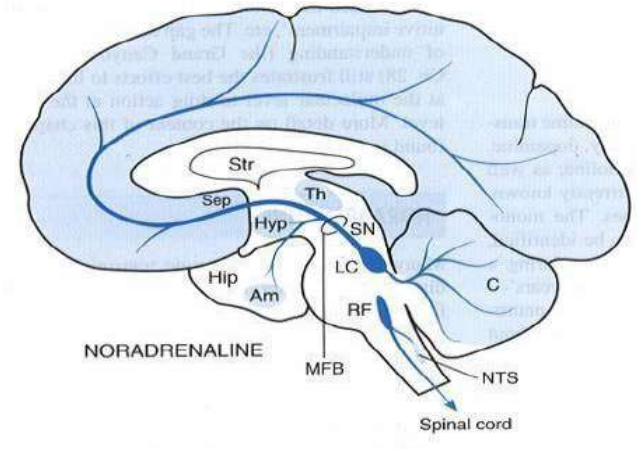


Figure 5: Noradrenaline pathways in brain[76]

(Am, amygdaloid nucleus; C, cerebellum; LC, locus ceruleous; Hip, hippocampus; Hyp, hypothalamus; MFB, medial forebrain bundle; NTS, nucleus of tractus solitarious; RF, brainstem reticular formation; Sep, septum; SN, substantia nigra; Str, corpus striatum; Th, Thalamus)

3.6.2 Serotonergic pathway in the CNS

The distribution of serotonergic neuron (Fig. 6) is similar to that of noradrenergic neurons. The cell bodies of those neurons are present in the raphe nuclei, pons, and upper medulla. The rostrally situated nuclei project via the medial forebrain bundle to many parts of the cortex, hippocampus, basal ganglia, limbic system and hypothalamus. The caudally situated cells project to the cerebellum, medulla, and spinal cord.

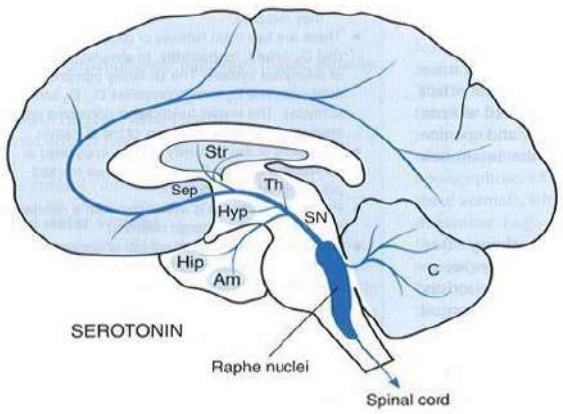


Figure 6: Serotonin pathways in brain[77]

(Am, amygdaloid nucleus; C, cerebellum; LC, locus ceruleous; Hip, hippocampus; Hyp, hypothalamus; MFB, medial forebrain bundle; NTS, nucleus of tractus solitarious; RF, brainstem reticular formation; Sep, septum; SN, substantia nigra; Str, corpus striatum; Th, Thalamus)

3.6.3 Dopaminergic pathway in the CNS

The antidepressants act by downregulation or desensitization of presynaptic autoreceptors.This increased release of neurotransmitters in the synaptic cleft which desensitize or 'down-regulate' the post-synaptic receptors thus lift the depression.[78]

In the central nervous system,dopamine-containing circuit regulates concentration, psychomotor speed, motivation and the ability to experience pleasure. Depression leads to impairment of these functions.

Dopamine present most abundantly in the corpus striatum is concerned with the movement coordination. Hypothalamus and limbic system also contain high concentrations of dopamine. Dopamine synthesis follows the path similar to norepinephrine but dopaminergic neurons lack dopamine β-hydroxylase and thus do not produce norepinephrine.

The midbrain dopamine neurons have three component pathways:

- The nigrostriatal pathway (from the substantia nigra to the corpus striatum) accounting for 75% dopamine in the brain. The cell bodies present in substantia nigra whose axon terminated in corpus striatum.
- The mesolimbic/mesocortical pathways whose cell bodies occur in the midbrain ventral tegmental area (VTA) and project to the limbic system such as nucleus accumbens and the amygdaloid nucleus and frontal cortex via the medial forebrain bundle.
- The turbo infundibular pathway is a group of short neurons running from the ventral hypothalamus to the median eminence and pituitary gland, the secretions of which they regulate.[79]

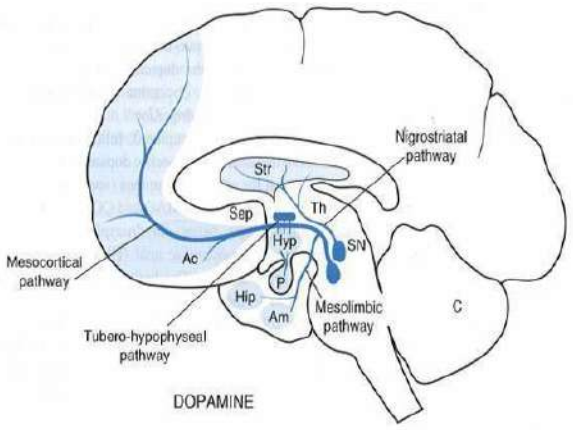


Figure 7: Dopamine pathways in brain[80]

(Am, amygdaloid nucleus; C, cerebellum; LC, locus ceruleous; Hip, hippocampus; Hyp, hypothalamus; MFB, medial forebrain bundle; NTS, nucleus of tractus solitarious; RF, brainstem reticular formation; Sep, septum; SN, substantia nigra; Str, corpus striatum; Th, Thalamus. P, pituitary gland; Ac, nucleus acumens)

3.7 Pathophysiology

The depression is a common and complex disorder of unknown etiology, numerous brain organs, systems and neuronal networks involved in maintaining the normal functioning of the body. Many research attempts to explain the mechanisms responsible for the development of depression and its treatment.

3.7.1 Neurodegenerative hypothesis

Several hypotheses of the depression have emerged over the past century

3.7.1.1 Biogenic amine hypothesis

Historically monoamine oxidase inhibitors were used as first-line antidepressants introduced in the late 1950s when isoniazid and iproniazid (hydrazine derivatives) drugs that were initially developed for the treatment of tuberculosis were reported to have mood-elevating effects in patients with tuberculosis and depression.[81]

The evolution of the monoamine hypothesis was the result of several observations made in the early 1950s. It was reported that the reserpine causes the depletion of dopamine, norepinephrine, and 5-hydroxytryptophan from vesicles of the neuron.[82]

According to the monoaminergic theory of depression, the majority of the antidepressant molecules that have been developed in the past were aimed at increasing extracellular levels of biogenic amines within the brain. Monoamine hypothesis the first neurochemical theory of depression stated that the depression is due to the deficiency of neurotransmitters such as serotonin, dopamine, norepinephrine and subnormal functioning of the receptor in a certain area of the brain.[83] The subsequent development of monoamine oxidase inhibitors was based on a similar approach namely an indirect elevation of extracellular concentration of the biogenic amines.

Since 30 years, monoamine hypothesis has become a keystone of research on depression. The effect of a tricyclic antidepressant and monoamine oxidase inhibitor on neurotransmission turnover are rapid, generally occurring within a few hour.[84] Still, the clinical relief takes much longer 6 to 8 weeks.

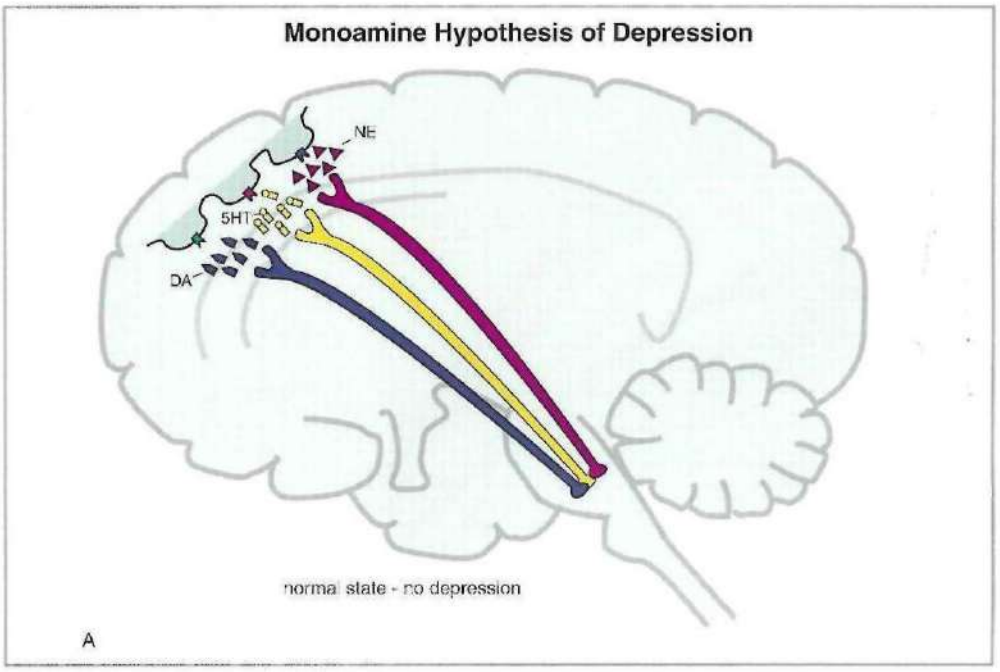


Figure 8: Classic monoamine hypothesis of depression, part 1[85]

According to the classic monoamine hypothesis of depression, when there is a "normal" amount of monoamine neurotransmitter activity, there is no depression present.

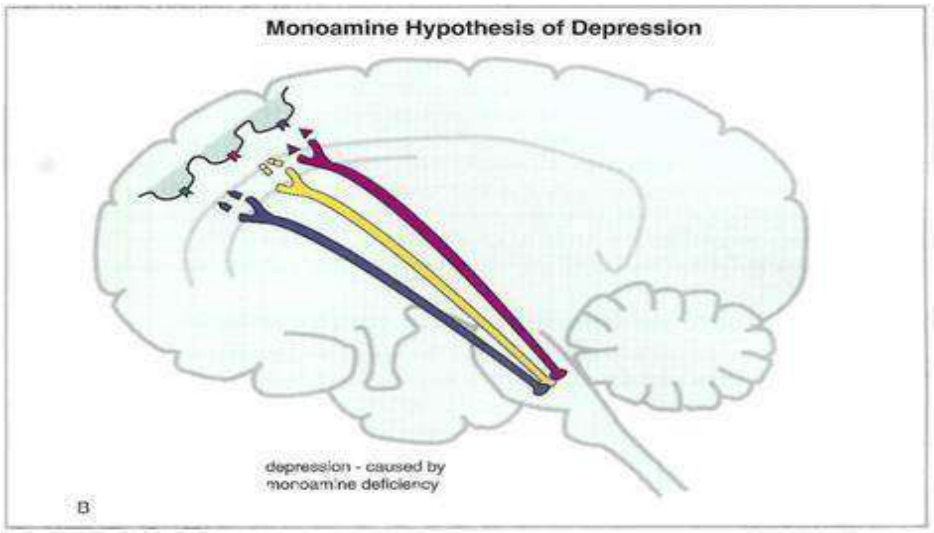


Figure 9: Classic monoamine hypothesis of depression, part 2[85]

The monoamine hypothesis of depression proposed that if the "normal" amount of monoamine, neurotransmitter activity becomes reduced, depleted, or dysfunctional for some reason, depression may ensure.

Table 5: Pharmacological evidence supporting the monoamine hypothesis of depression.[87]

Drug(s)	Principal action	Effect in depressed patients
Tricyclic antidepressants	Block NE and 5-HT reuptake	Mood ↑
Monoamine oxidase (MAO) inhibitors	Increase stores of NE and 5-HT	Mood ↑
Reserpine	Inhibits NE and 5-HT storage	Mood ↓
α-Methyltyrosine	Inhibits NE synthesis	Mood↓(calming of manic patients)
Methyldopa	Inhibits NE synthesis	Mood ↓
Electroconvulsive Therapy	Increases central nervous system responses to NE and 5-HT	Mood ↑
Tryptophan (5-hydroxytryptophan)	Increases 5-HT synthesis	Mood ↑ in some studies

3.7.1.2 The Receptor sensitivity hypothesis`

A more perplexing aspect of the observed effects of antidepressants is the discrepancy between monoamine reuptake blockade (immediate) and any measurable improvement in depressive symptoms (delayed therapeutic response). In depression, decrease in neurotransmitters in synaptic cleft lead to increase the receptor response by compensatory mechanism and is called as up-regulation.

Antidepressant drugs act by increasing the level of neurotransmitter in the synaptic cleft. The chronic administration of antidepressant drugs decreases the sensitivity of norepinephrine-stimulated cyclic AMP synthesis. In fact for most antidepressant downregulation of β adrenergic receptors accompanies this desensitization.[88] Researchers reported the down-regulation of serotonin and norepinephrine receptors fallowing chronic administration of antidepressants.[89]

Thus, a theory based on postsynaptic changes in receptor sensitivity provides a cogent explanation of the delayed onset of activity of antidepressant drugs.[90]

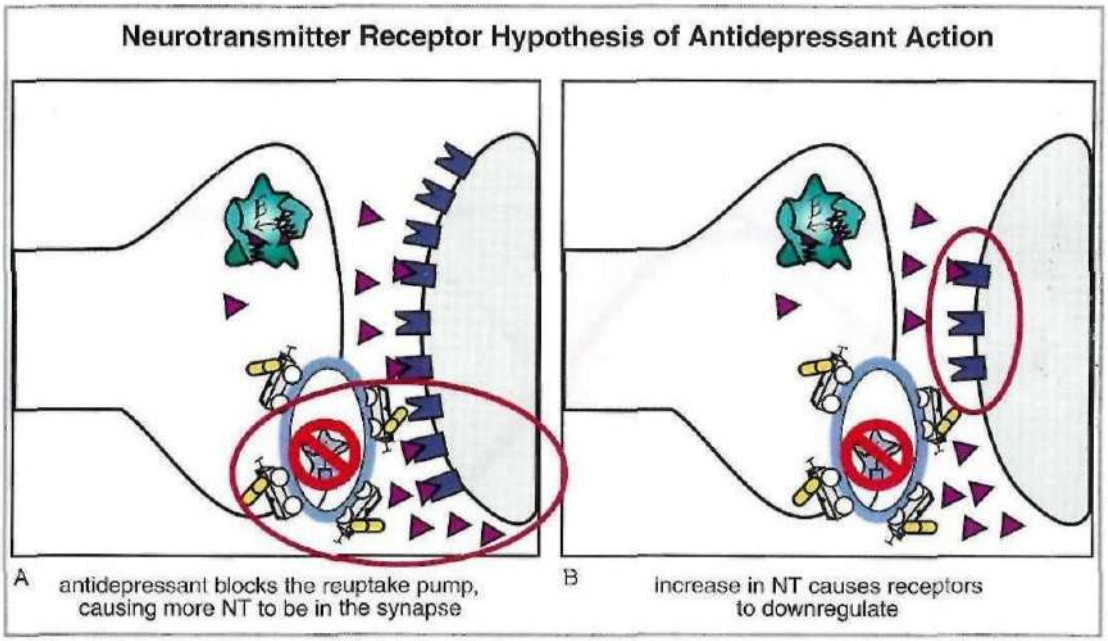


Figure 10&11: A and B Neurotransmitter receptor hypothesis of antidepressant action[91].

Although antidepressants cause an immediate increase in monoamines, they do not have immediate therapeutic effects. This may be explained by the monoamine receptor hypothesis of depression, which states that depression is caused by upregulation of monoamine receptors; thus antidepressant efficacy would be related to downregulation of those receptors, as shown here. (A) When an antidepressant blocks a monoamine reuptake pump, this causes more neurotransmitter (NT) (in this case, norepinephrine) to accumulate in the synapse. (B) The increased availability of NT ultimately causes receptors to downregulate. The time course of receptor adaptation is consistent both with the delayed clinical effects of antidepressants and with the development of tolerance to antidepressant side effects.

3.7.1.3 5-HT/NE link hypothesis

It is evident that no single neurotransmitter theory of depression is adequate. The major depressive disorder (MDD) is the result of disturbances in the limbic and cognitive area of the brain where NE and 5HT overlaps. Thus the combination of serotonin-norepinephrine reuptake inhibitors (SNRIs) may be more effective than a single drug. 5HT regulates NE neurons and reciprocally that NE also regulates 5HT neurons. Thus, both NE and 5HT has bidirectional control.[92]

This hypothesis is inconsistent with the postsynaptic alteration theory of depression which emphasizes the importance of α- adrenergic receptor downregulation and β-adrenergic receptors desensitization for achieving antidepressant activity.[93]

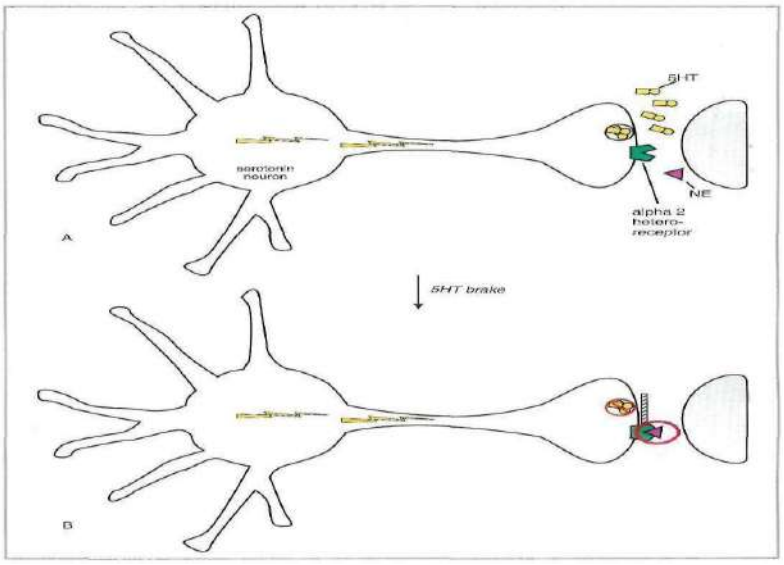


Figure 12&13: A and B Norepinephrine as a brake on serotonin release.[94]

Alpha 2 adrenergic heteroreceptors are located on the axon terminals of serotonin neurons. When these receptors are unoccupied by norepinephrine, serotonin is released from the serotonin neuron (A). However, when norepinephrine binds to the alpha 2 receptor this closes the molecular gate and prevents serotonin from being released (B).

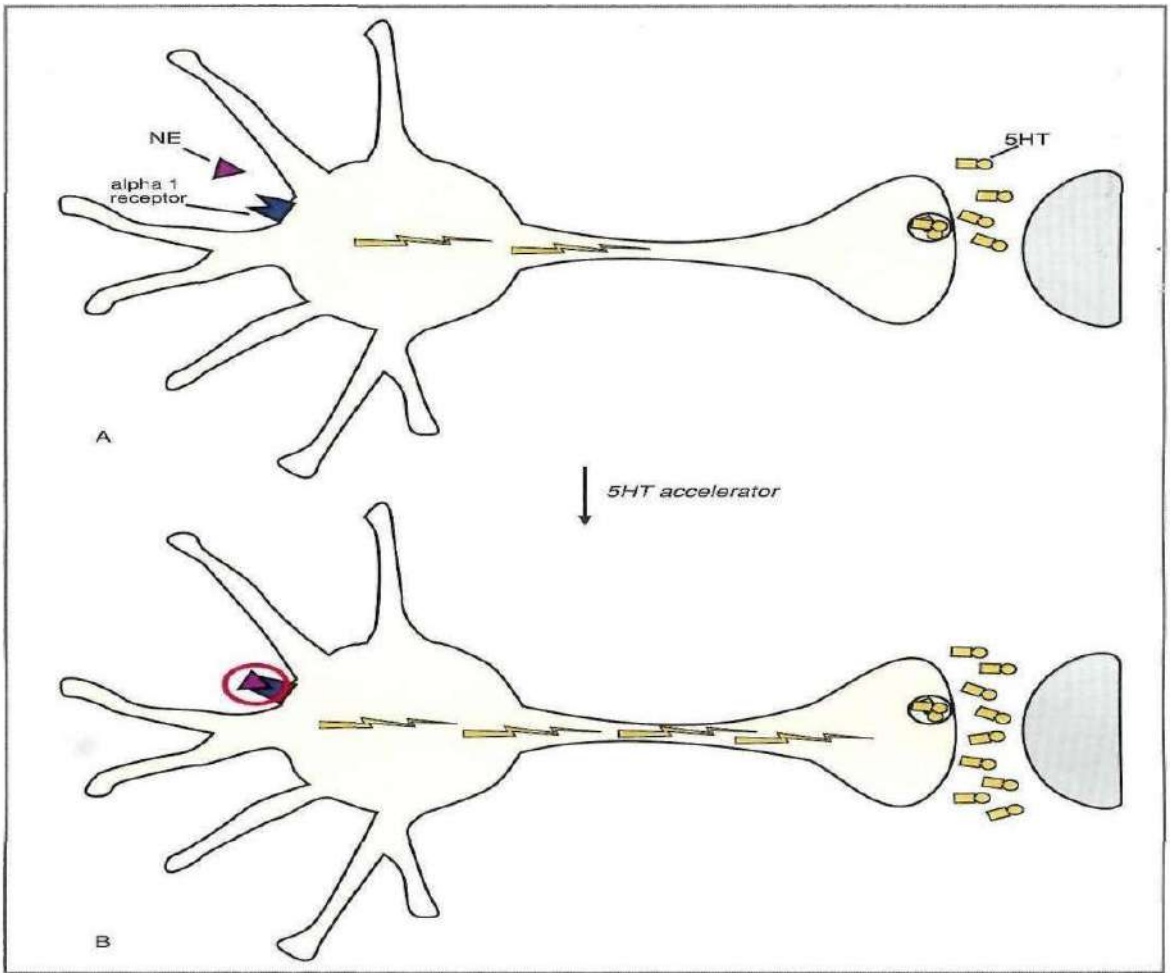


Figure 14&15: C and D **Norepinephrine as an accelerator of serotonin release.**[95] Alpha 1 adrenergic receptors are located in the somatodendritic regions of serotonin neurons. When these receptors are unoccupied by norepinephrine, some serotonin is released from the serotonin neuron (C). However, when norepinephrine binds to the alpha 1 receptor this stimulates the serotonin neuron, accelerating the release of serotonin (D).

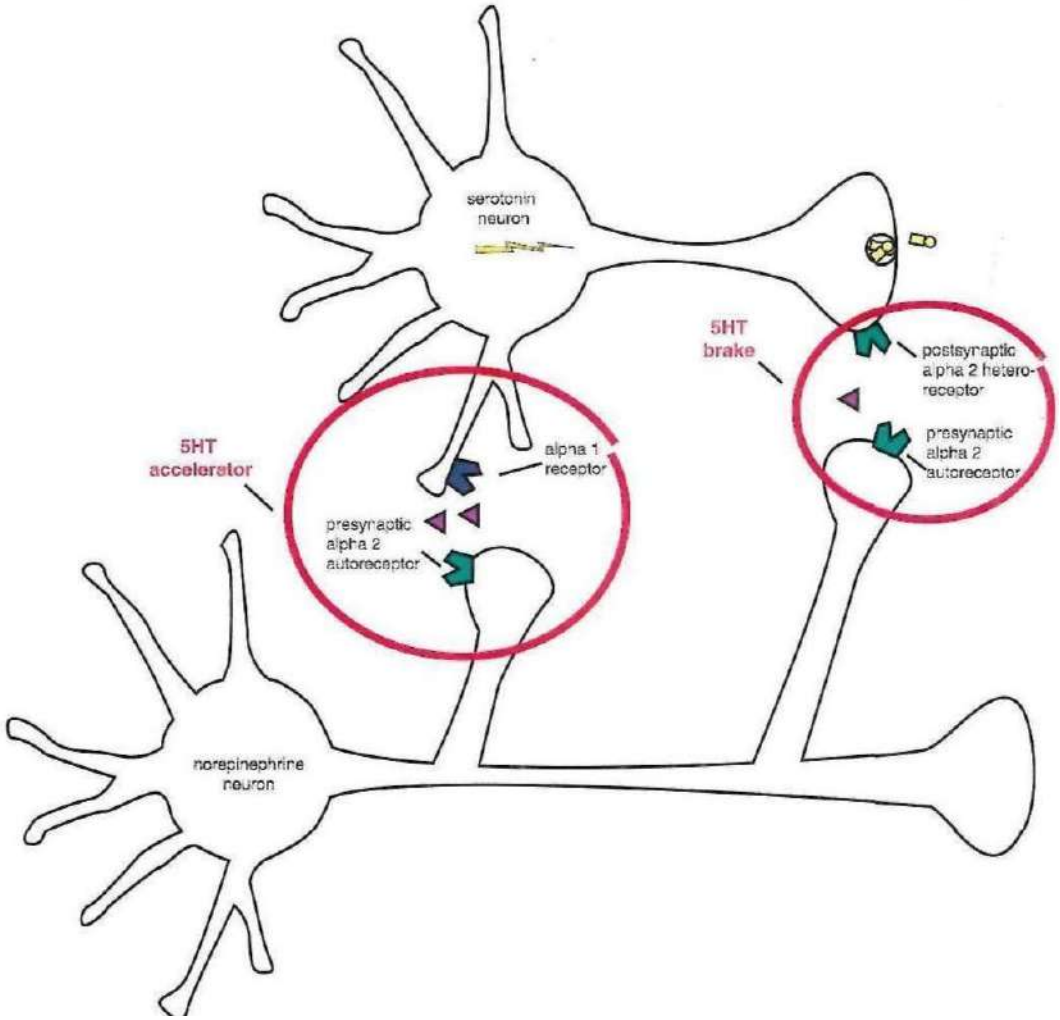


Figure 16: Norepinephrine bidirectional control of serotonin.[96]

Norepinephrine can act as a brake on serotonin release when it binds to alpha 2 receptors at the axon terminal and as an accelerator of serotonin release when it binds to alpha1 receptors at somatodendritic regions. Thus, norepinephrine has bidirectional control of serotonin release.

3.7.1.4 HPA axis hyperactivity hypothesis

In depression, hypothalamic-pituitary-adrenal axis and hypothalamic-pituitary-thyroid- axis play an important role. The hyperactivity of HPA axis and defective HPA-axis glucocorticoid feedback mechanisms are widely reported in the neurobiology of depression.[97]

In addition, patients with major depression have been shown to exhibit increased concentrations of cortisol in cerebrospinal fluid, plasma and urine[98] and exhibit an

exaggerated cortisol response to adrenocorticotropin hormone (ACTH).[99] There is evidence that administration of CRF has been shown to induce behavioral changes in animals that are comparable to those seen in human depression (e.g. alterations in mood, appetite, sleep, locomotor activity and cognition).[100] In depression, increased CRF release from paraventricular nucleus of the hypothalamus is due to the failure of cortisol to suppress CRF secretion due to the failure of negative feedback mechanism.[101]

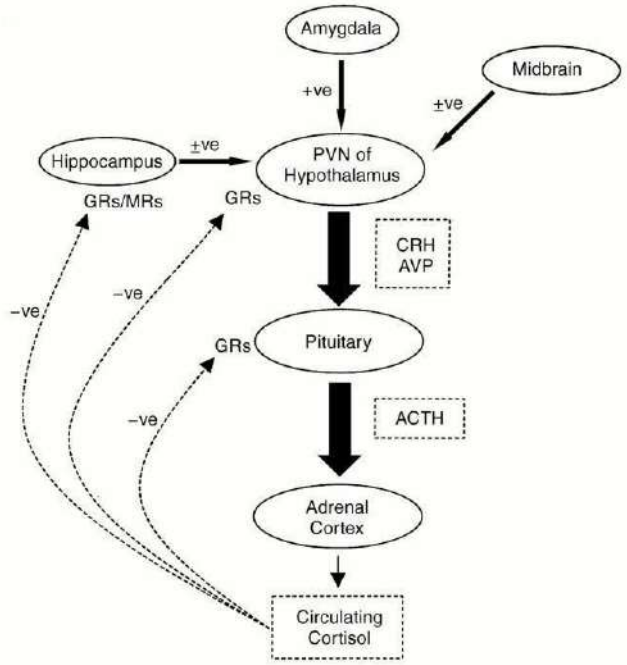


Figure 17: Negative feedback regulation of cortisol[102]

3.7.1.5 Cholinergic hypothesis

The cholinergic system is responsible for a number of CNS functions including arousal, attention, learning, and memory. The depression is often associated with cognitive impairments, mood disturbances and other autonomic, endocrine and sleep-wake abnormalities.

The cholinergic-adrenergic imbalance theory described in early 1970 suggested that an overactivity of the cholinergic system causes depression.[103] The cholinergic hypothesis suggested that the cholinergic neurons are generally antagonistic to catecholaminergic neurons and led to the cholinergic-noradrenergic imbalance.[104] This suggested that an imbalance between these systems resulted in depression.

The acetylcholine (ACh) significantly mediates the neuroendocrine and physiological responses to stress and facilitates the release of several stress-sensitive neurohormones and peptides including corticotrophin releasing factor (CRF), ACTH.[105] Janowsky and co-workers in 1994 reported that cholinergic hyperactivity and genetic predisposition may be the marker for an affective disorder. It is observed that central choline (precursor to acetylcholine) level is increased in the depressed patient as compared to normal control.[106] Neuroendocrine effect of acetylcholine is mediated through activation of nicotinic acetylcholine receptor nAChRs.[107] In the hypothalamus, Ach induces the CRF release, which is inhibited by the nAChR blockade. The antidepressant may reduce the symptom of depression through blockade of nAChRs involved in stress-induced activation of HPA axis.[108]

3.7.1.6 Immunological hypothesis

An association between immunological alteration and depression is described for over 2 decades.[109] The researcher has shown that immune activation and production of cytokines may be involved in depression.[110] Cytokines are small cell-signaling proteins produced by astrocytes, microglia, and a neuron that mediate and regulate immune responses and inflammations. The cytokines are of two types viz pro-inflammatory and anti-inflammatory. Helper T-cell type 1 (Th1) produced pro-inflammatory mediators such as INF ϒ , TNF, IL 1, IL 2 and Helper T-cell type 2 (Th2) produced anti-inflammatory mediators(IL 4, IL 5 and IL 10) which inhibit the helper T cell response (Th 1).The balance between pro-inflammatory and anti-inflammatory cytokine is essential to prevent excess inflammation. The imbalance between helper T-cell type 1 (Th1) and helper T-cell type (Th2) leads to increase in the level of proinflammatory cytokines like IL-1, IL2, IL-6 and TNF- α. in plasma and cerebrospinal fluid play important role in the development of depression.

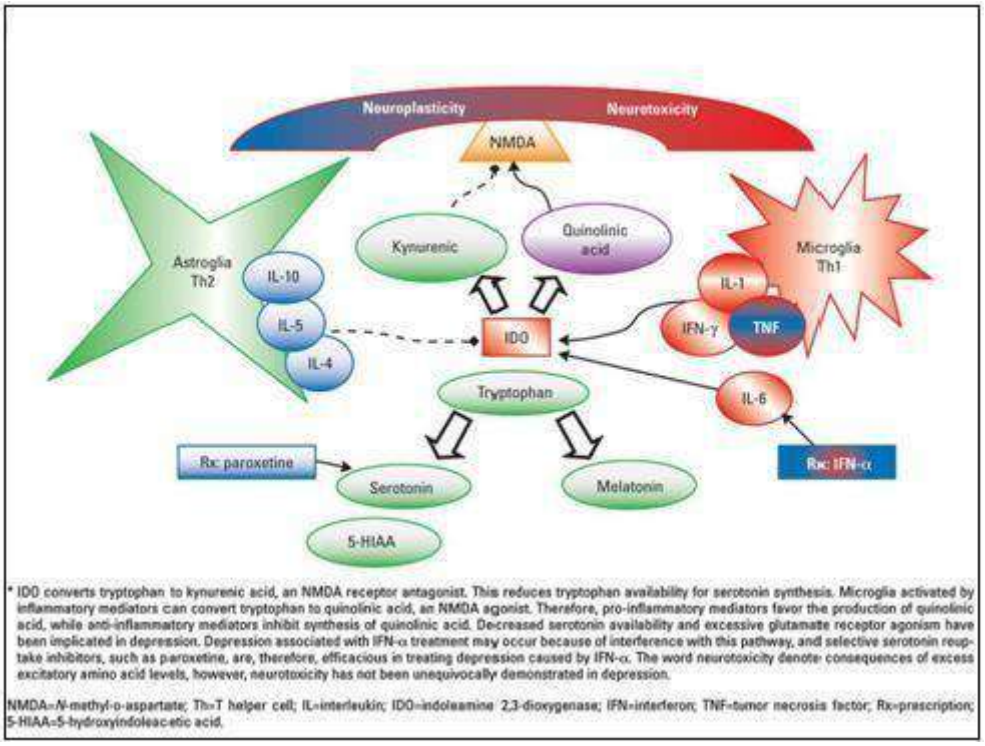


Figure 18: Tryptophan, kynurenine and quinolinic acids[111]

3.7.1.7 Neuroplasticity hypothesis

A neuron is the fundamental unit of the central nervous system. Its function is to receive, store and process information. The neuronal plasticity concern with strengthening or weakening nerve connections that are the ability of neurons and neural elements to adapt in response to internal and external signals. The researcher reported that chronic stress results in the reduction of dendritic spines in the prefrontal cortex, length, and complexity of cortical dendrites, volumetric reductions and cell death/atrophy of some neural cells such as hippocampal CA3 pyramidal neurons and impair neurogenesis observed in mood disorder.[112]

Several mechanisms of synaptic plasticity reported that synaptic calcium influx and second messenger cyclic AMP triggered synaptic potentiation. Serotonin, norepinephrine, dopamine and calcium regulate the level of cyclic AMP. Therefore decreased neurotransmission in depressed patient disturb the neural plasticity. Calcium and cAMP-induced short-term synaptic plasticity by activation of protein kinase-A (PKA) and calcium–calmodulin-dependent kinase II (CaMKII). Thus, calcium-calmodulin kinase contributes to both long term and short term potentiation.

LTP is a process in which synapses are strengthened.[113]

It is evident that chronic stress and mood disorder disturbed the neuronal plasticity.[114] Chronic stress results in hyperactivity of hypothalamic pituitary adrenal (HPA) axis that results in the abnormal production of glucocorticoids, an elevated level of which suppresses the hippocampal neurogenesis.[115] Therefore, dysregulation and/ or disruption of neural plasticity and increased glucocorticoids level are associated with the neurodegenerative and neuropsychiatric disease. Antidepressants may act by enhancing neuroplasticity mechanisms and increases hippocampal neurogenesis by renewing the impairment in neural circuits contributing to their normalization and may prevent stress-induced hippocampal neuron atrophy.[116]

3.7.1.8 Neurotrophic hypothesis of depression

The majority of studies evaluating the role of neurotrophins in depression focused on the role of BDNF, a biomarker of depression.The neurotrophic hypothesis of depression state that the reduction in BDNF leads to atrophy of neuron, decrease hippocampal neurogenesis and loss of glia cells. Antidepressant act by reversing the decreased. BDNF defect.[117] It is evident from the clinical, preclinical and brain imaging studies that acute and chronic stress leads to a reduction of neurotrophic factor (BDNF) in dented gyrus and pyramidal cells of the hippocampus and prefrontal cortex in rodent models, whereas chronic antidepressant treatment restores BDNF expression and increases the dendritic arborization/formation in this region.[118] Various studies demonstrate and propose strong evidence of strategies used–infusion of BDNF that produces antidepressant activity in the behavioral model.[119] Several systems which involve in depression like serotonin and norepinephrine neurotransmitter system, BDNF is reported to be a potent neurotrophic factor. It produces its effect by influencing the monoaminergic systems either by acting on the presynaptic system that increases the function of monoamine neuron or postsynaptic sites by increasing the output of target neuron.[120] It also involves in adult neurogenesis of hippocampal cells. It has been demonstrated that antidepressant drugs produced its action by regulation of CREB, thus ultimately increases the levels of neurotrophic factors in the hippocampus, such as BDNF and VEGF.[121]

3.7.1.9 GABA and Glutamate hypothesis

The GABAergic theory of depression was raised in the 1990s. GABA is an inhibitory neurotransmitter in the brain and regulates norepinephrine and dopamine turnover. Glutamate constitutes 50-60% of all neurotransmission in the brain and the remaining 40-50% is GABAergic[122] and less than 10% is left for all the others monoamines, neuropeptides, and neuroendocrine neuromodulators. The fundamental aspect of the proper functioning of the CNS is keeping the excitatory/inhibitory physiological balance.[123] Any disruptions within this balance may lead to a brain dysfunction reflected as a mental disorder.

Biochemical studies confirmed the involvement of GABAergic mechanisms in mood disorders, as it was hypothesized that antidepressant drugs may act through increasing the GABAergic tone. The up-regulation of GABAB receptors appeared to be the fundamental facet of antidepressant drug action.[124] Parallel to these observations it was shown that the level of GABA was decreased in the plasma of depressed patients and the level of Glutamate decarboxylase **(**GAD67), enzyme synthesizing GABA from glutamate, was lowered in the brains of those patients. Recent studies confirmed the importance of both GABA receptors in depression, suggesting GABAB neurophysiological deficits to be related to the pathophysiology of major depressive disorder.[125]

3.7.1.10 Dopamine hypothesis

The original hypothesis of dopamine which was formulated in 1996 by Van Rossum suggested that the overactivity of dopamine pathways in the brain was responsible for the disorder and linked schizophrenia and psychosis with dopamine (DA) activity.[126] Subsensitisation of presynaptic DA receptor occurs after chronic treatment of antidepressant which results in increased DA release.[127]

Several pieces of evidence suggest that increased dopamine neurotransmission in the nucleus accumbens may represent a part of the mechanism of action of antidepressant medications.[128]

The biochemical evidence derives from studies of homovanillic acid, a dopamine metabolite, indicating diminished dopamine turnover in patients with depression.[129]

3.8 Biologic markers

Biomarkers are "biological clues" that are used in medicine to confirm the presence or risk of diseases. Thus Also, provide indicators of normal biological processes, pathogenic processes or pharmacological responses for the treatment of medical diseases. These clues are usually found in changes in blood, urine or body tissue.

For the recognition, understanding and treatment of major depression researchers continue to search the biological or pharmacodynamic markers. Although among several existing biological abnormality in depressed patients around 45%-60% of patients are associated with a neuroendocrine abnormality such as stress-related alterations in cortisol level (due to dysregulation of the hypothalamic-pituitary-adrenal axis).

The dexamethasone suppression test is the most specific measure of hypothalamic-pituitary-adrenal axis overactivity.[130] Immune system dysregulation contributes to symptoms of major depression[131]. Specifically, increases in the production of pro-inflammatory cytokines linked with depressive behaviors.

Brain-derived neurotrophic factor (BDNF) plays an important role in the pathogenesis of major depression since altered BDNF-dependent signaling is observed in the brain of depressed patients.

3.9 Antidepressant drugs

These are the drugs involved in elevation of mood in a depressed patient.[132]

Classifications:-

I. **Monoamine oxidase inhibitors (MAOI**)

- Selective MAO inhibitors
- Non-selective MAO inhibitors e.g. Phenelzine, tranylcypromine, iproniazide, iso-carboxazide

II. Inhibitors of monoamine uptake

Tricyclic antidepressant (TCA)

5HT–NE reuptake inhibitors e.g. Imipramine, amitriptyline

NE reuptake inhibitors e.g. Desipramine, nortryptyline

III. **Selective 5-HT (serotonin) reuptake inhibitors (SSRI)**
E.g. Fluoxetine, fluvoxamine, paroxetine, sertraline

IV. **Atypical antidepressants**: these are compounds with non-selective receptor-blocking effects and their antidepressant actions are poorly understood e.g. Mianserin, bupropion, and trazodone

Table 6: Types of antidepressant drugs and their characteristics

Type and examples	Action	Unwanted effects
Monoamine oxidase Inhibitors(MAOIs)	Inhibit MAO-A and/or MAO-B, Non-selective	„Cheese reaction" to tyramine containing foods, anticholinergic side effects
Phenelzine, isocarboxazid, Tranylcypromine, Moclobemide	MAO-A selective short-acting	hypotension, weight gain, liver damage, Nausea, insomnia, agitation.
Monoamine uptake Inhibitors Tricyclic (TCA)	Inhibitor of NE/5-HT reuptake	Sedation, Anticholinergic effects (dry mouth, constipation, blurred vision, urinary retention, etc.), postural hypotension, seizures, impotence, interaction with CNS depressants (alcohol, MAOIs)
Imipramine, amitriptyline	NE reuptake inhibitors	As above
Desipramine, nortriptyline	NE reuptake inhibitors	As above
Selective serotonin reuptake inhibitors (SSRI)		Nausea, diarrhea, agitation, insomnia, anorgasmia, inhibit the metabolism of other drugs
fluoxetine, fluvoxamine paroxetine, sertraline	All highly selective for 5-HT	
Atypical antidepressants		

1.Bupropion **2. Trazodone** **3. Mirtazapine**	Weak dopamine and 5-HT uptake Inhibitor. blocks 5-HT-2 and 5HT1-receptors(enhances NE/5-HT release) Blocks α2, 5-HT2- and 5-HT3-receptors	Dizziness, anxiety, Seizures. Sedation, hypotension, cardiac dysrhythmias, dry mouth, sedation, weight gain
Miscellaneous Maprotiline, reboxetine, Venlafaxine St. Johns wort (active principle : hyperforin)	Selective NE uptake inhibitor weak non-selective NE/5-HT uptake inhibitor, also non selective receptor-blocking effects Weak non- selective NE/5-HT uptake inhibitor, also a non – selective receptor blocking effects	As TCA, no significant advantages, dizziness, insomnia, anticholinergic effects as SSRIs, withdrawal effects common and troublesome if dose is missed Few side-effects reported

.9.1 Monoamine oxidase inhibitors (MAOI)

The first drug which was introduced clinically as antidepressant was monoamine oxidase inhibitor. These drugs inhibit monoamine oxidase enzyme. The enzyme is existing in two molecular forms i.e. MAO-A and MAO-B. New generation antidepressant involves reversible inhibitor of monoamine oxidase.[133]

3.9.1.1 Mechanism of action

Monoamine oxidase (MAO) the principle enzyme responsible for the regulation and the catabolism of monoamines has been found to be involved in the depression. It is located in the outer mitochondrial membrane of many tissues. MAO-A preferentially involves in metabolism of serotonin and norepinephrine while MAO-B involves in the metabolism of phenylethylamine and benzylamine. Dopamine is

metabolized equally by both MAO-A and MAO-B.

Nerve terminals of serotonin and norepinephrine, intestinal mucosa and human placenta contain mainly MAO-A; whereas certain areas of brain and blood platelets contain MAO-B. Liver contains both isoenzymes. Inhibition of MAO-A than MAO-B is responsible for the treatment of depression. Inhibition of this enzyme on the administration of MAO inhibitor (MAOI) leads to increase the concentration of monoamines in central nervous system.[134]

3.9.1.2 Unwanted effects

The unwanted effects of MAOI include hypotension, weight gain, dry mouth, blurred vision and urinary retention. Excessive central stimulation leads to tremors, excitement, insomnia and in overdose, convulsions and MAOI of hydrazine type (phenelzine and iproniazid) produce, very rarely, severe hepatotoxicity. Their use in patients with liver disease is, therefore, unwise.[135]

3.9.1.3 Interactions with food and other drugs

1. Cheese reactions- Tyramine is an indirectly acting sympathomimetic amine which is actively transported to the sympathetic nerve ending through the neuronal reuptake mechanism.[136] It acts by releasing neurotransmitter norepinephrine (NE) from the adrenergic axonal terminal, which in turn increases the systolic blood pressure.[137]

The ingestion of monoamine oxidase inhibitors with tyramine-rich food and beverages such as beer, red wine, aged cheeses, broad beans, yeast extract, soy sauce, banana peels, meat extract and buttermilk causes hypertensive crises.[138] The factors contribute to a potentiation of pressor effect of tyramine are-

- Normally tyramine is metabolized in the liver by MAO enzymes. MAOI by inhibiting detoxification lead to tyramine accumulation which results in the release of norepinephrine from binding sites causing a marked rise in blood pressure.
- Metabolic inactivation of tyramine took up into sympathetic nerve ending and
- Prevention of displaced norepinephrine deamination.[139]

In recent year reversible inhibitor of monoamine oxidase -A e.g. Moclobemide has

been reported safer in terms of hypertensive crises than older MAOI.

3.9.1.4 Uses-

Nonselective MAO inhibitors are rarely used in a major depression in the patients not responding to tricyclic antidepressant and in a patient for whom electroconvulsive therapy (ECT) is contraindicated or refuse. Certain neurotic problems like phobic states, obsessive-compulsive behavior respond favorably to MAO inhibitors.

They are helpful in narcolepsy by inhibiting rapid eye movement (REM) sleep.

Moclobemide is a reversible, selective MAO inhibitor that weakly potentiate the pressor response to tyramine .It lacks the anticholinergic, sedation, cardiovascular effect of TCA thus emerging as a good alternative to TCAs for major depression and social phobia.

Use in treatment of a migraine, bipolar depression, psychotic depression, unipolar endogenous depression.

3.9.2 Tricyclic antidepressant drugs (TCA)

TCA is one of the important class, but far from ideal in practice.

3.9.2.1 Mechanism of action

TCA inhibit the neuronal uptake by competitively binding to transport protein. Synthesis storage and release of neurotransmitter is not affected directly but some TCA act indirectly by increasing the release of neurotransmitter by acting on presynaptic α_2 –adrenoceptors. It inhibited 5HT and noradrenaline uptake but showed less effect on dopamine uptake. Enhancement of 5HT -mediated transmission improved the emotional symptom whereas facilitation of noradrenergic transmission resulted in relief of biological symptoms. TCAs metabolites have different pharmacological activity than parent compounds.[140]

TCAs other than amine uptake also act on neurotransmitter receptors such as histamine, muscarinic acetylcholine and 5-HT receptors. They have a moderate and selective affinity for α_1-adrenergic receptors, much less for α_2 and virtually none for β- receptors. The longer onset of action may be due to the α_2 receptor-mediated negative feedback mechanisms rapidly activated by administration of tricyclic antidepressants.

3.9.2.2 Unwanted effects

TCA produces unwanted effects that include, weight gain, mental confusion, sedation, weakness, anticholinergic,dry mouth, blurred vision, constipation, bad taste, epigastric distress, urinary retention and palpitation. Sweating and fine tremors are also noted with most TCA. Some patients may switch to hypomania or mania, seizure, postural hypotension (in older patients) and cardiac arrhythmias.[141]

3.9.3 Selective Serotonin Reuptake Inhibitors (SSRI)

To conquer the shortcomings of TCA, newer antidepressants have been developed since the 1980s. The newer drugs have suppressed older TCAs in overall efficacy and tolerability. Thus, it becomes the first-line drug in the treatment depression due to their safety and acceptability.[142]

3.9.3.1 Mechanism of action

Mechanism of action of serotonin consists of 3 steps

Step 1-Before treatment

The depressed patient shows a deficiency of 5-HT in the synaptic cleft which result in upregulation of posts synaptic and presynaptic autoreceptors. Serotonin release into the synaptic cleft is influenced either by the availability of serotonin precursor eg. Tryptophan or by activation of the inhibitory presynaptic receptor which results in increase the neuronal firing that leads to increasing in serotonin release in the synaptic cleft.

Step 2-acute administration of SSRI

Selective serotonin reuptake inhibitor blocks the serotonin transporters. This causes the concentration of serotonin increase initially at the somatodendritic area and not in the axon terminals. The inhibitory presynaptic and somatodendritic receptors mediate the negative feedback mechanism.

Step 3- chronic treatment with SSRI

The enhanced 5-HT concentration at the inhibitory somatodendritic receptors causes them to down-regulate and/or desensitize, which results in an increase of the frequency of firing of action potentials and in an increase of the amount of serotonin released into the synaptic cleft.

This delay in time is required to desensitize or downregulate the somatodendritic autoreceptors and turn on neuronal impulse flow in the serotonin neuron. This delay may explain why antidepressants take more time to relieve depression.

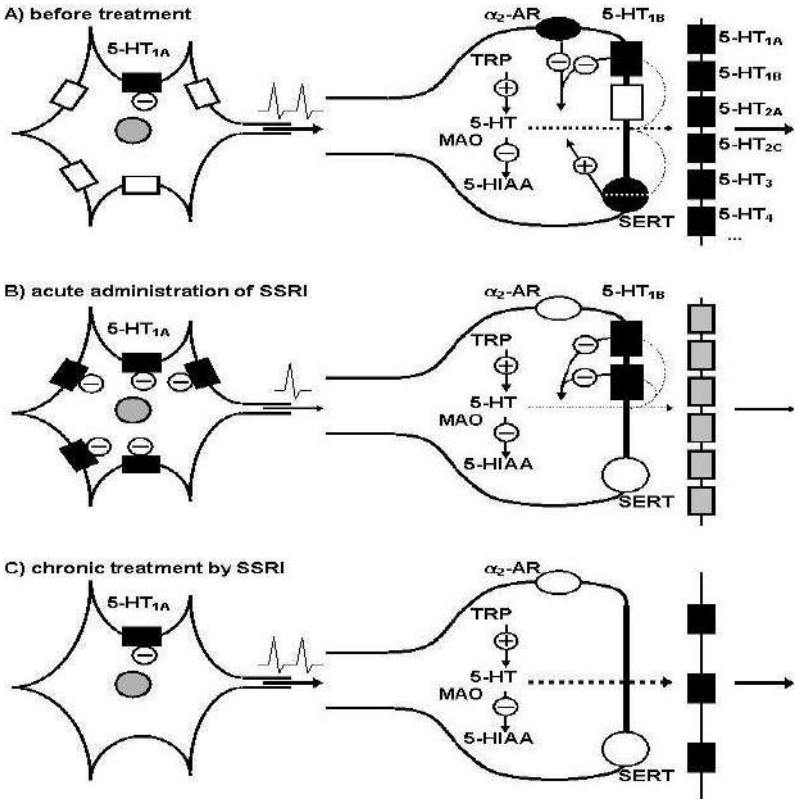


Figure 19: Mechanism of action of selective serotonin reuptake inhibitors (http://psych.lf1.cuni.cz/bpen/psychopharmacology.htm)

3.9.3.2 Unwanted effects

The SSRI produce comparatively less unwanted side effects. However, they frequently produce nausea, nervousness, insomnia, restlessness, anorexia, headache, dyskinesia and diarrhea.This drug may impair both hepatic and renal function and are contraindicated in patients with hepatic impairment. Another serious complication secondary to their effects in increasing serotonergic activity is serotonin syndrome.[143]

3.10 Literature survey and need of research work

Depression is recognized as the most common psychiatric illnesses with an estimated prevalence of 15–20% of the general population. Such numbers themselves justify the need for a more rapid treatment as a matter of comfort for millions of patients. In addition, the delayed onset of action of antidepressants becomes a critical factor in the particular cases of major/severe depressions, in which the risks of suicide are strongly increased.[144]

Considering the history of monoamine oxidase inhibitor safety is the most important factor from a clinical point of view in order to develop safe and effective antidepressants. In the present study we are using compound synthesized in our laboratories. Dr. Mugdha Suryawanshi, department of pharmaceutical chemistry, Poona college of pharmacy, Pune has synthesized two series of compounds, one series of compounds was synthesized through the pharmacophore study on 82 molecules and pharmacophore was developed using PHASE software. This pharmacophore was matched using ZINC database and out of 1,40,000 molecule, 11 hits were obtained.[145] From this 1 hit was selected and modified.

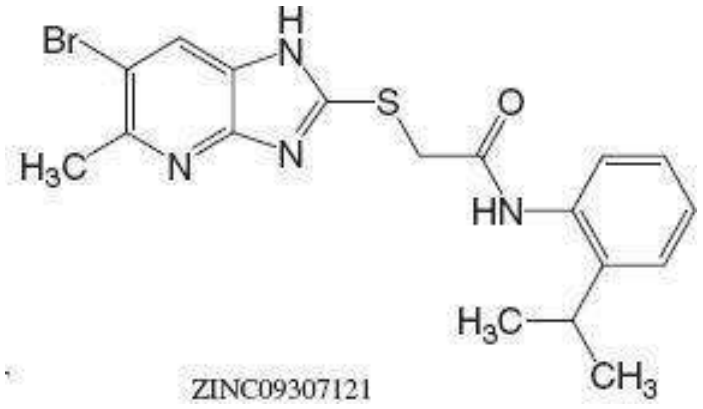


Figure 20: ZINC09307121

The designed molecule was subjected to docking study by using „Glide". Total 25 compounds were synthesized and characterized and the preliminary study was carried out using a small number of animals. From this one derivative which is significantly active i.e. 2[N-benzylacetamido) mercapto] benzimidazole (VS-25) was used in the present study.

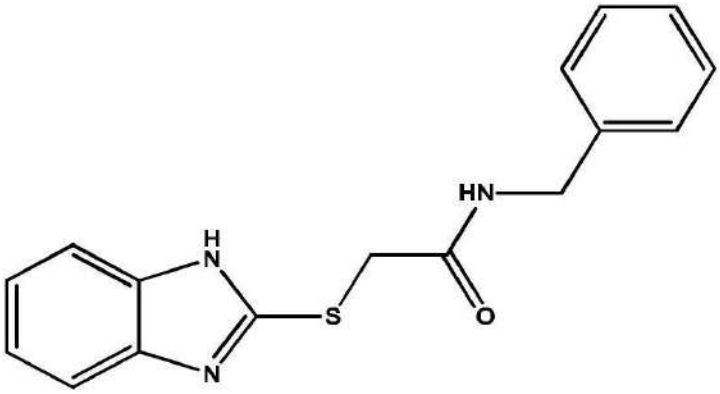


Figure 21: 2[N-benzylacetamido) mercapto] benzimidazole

Many antidepressant agents used in the clinical practice have a tricyclic structure. Therefore in the second series, two existing tricyclic molecules one having quinoxiline ring reported as a serotonin reuptake inhibitor and another oxadizino ring as a reversible monoamine oxidase inhibitor were fused together to get a new molecule which was subjected to docking study. Total 15 compounds were synthesized and characterized.[146]

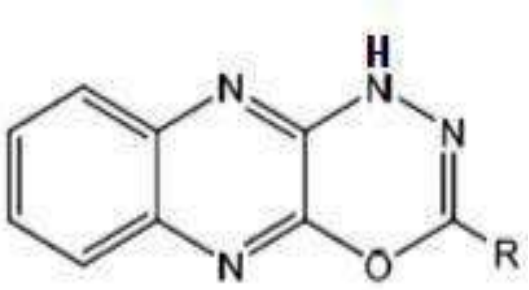


Figure 22: Substituted 1,3,4-oxadiazino [5,6-e] quinoxaline

The preliminary study was carried out and subjected to QSAR study for the confirmation of group responsible for the antidepressant activity. She has undertaken preliminary study (subchronic study) using small number of animals[147] and one derivative which was significantly active viz. 4-Acetyl-2-(4-nitrophenyl)1,3,4 oxadiazino[5,6-e] quinoxaline (VMS-15) was used in the present study

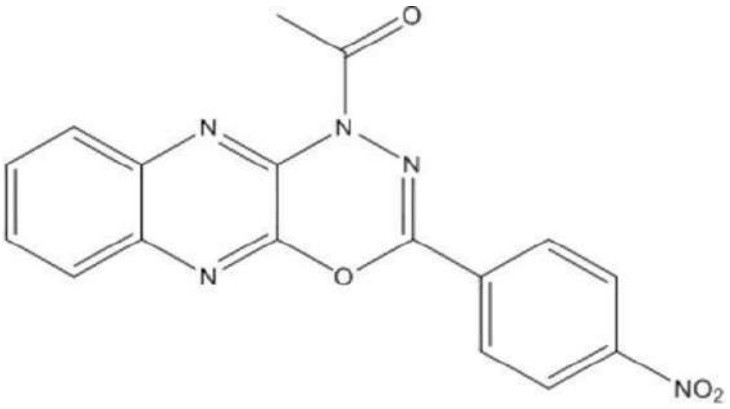


Figure 23: 4-Acetyl-2-(4-nitrophenyl)1,3,4 oxadiazino[5,6-e] quinoxaline

The results of ligand-based pharmacophore hypothesis and atom based 3D-QSAR gave structural insights as well as highlighted important binding features of MAO inhibitors and tricyclic derivatives as selective serotonin reuptake inhibitors.

Therefore, in the present study, one derivative from each series was selected for extensive pharmacological investigations.

3.11 Pharmacological screening of antidepressants.

The first antidepressant drugs were detected by serendipity in clinical trials. Iproniazid was developed for the treatment of tuberculosis. The observation of mood elevating effects was followed by the detection of the inhibition of the enzyme monoamine oxidase. During the clinical investigation of phenothiazine analogs as neuroleptics, imipramine was found to be relatively ineffective in agitated psychotic patients but showed remarkable benefit in depressed patients. Later on, inhibition of uptake of biogenic amines was found to be the main mechanism of action resulting in downregulation of β-receptors.[148] Influence on α_2-adrenoreceptors[149] was discussed as well. Several lines of preclinical and clinical evidence indicate that an enhancement of 5-HT-mediated neurotransmission might underlie the therapeutic effect of most antidepressant treatments.

3.11.1 Behavioral tests

3.11.1.1 Catalepsy antagonism in chicken

An observation about cataleptic behavior in chicken was first described more than 300 years ago[150] and again reported about 100 years ago.[151] This phenomenon was used by Vogel and Ther (1963) as a simple method to detect antidepressants besides other central stimulants.

3.11.1.2 Forced swim test

Behavioral despair was proposed as a model to test antidepressant activity by Porsolt *et al.*(1977, 1978)[152] it was suggested that mice or rats forced to swim in a restricted space from which they cannot escape are induced to characteristic behavior of immobility. This behavior reflects a state of despair which can be reduced by several agents which are therapeutically effective in human depression.

3.11.1.3 Tail suspension test in mice

The tail suspension test has been described by Steru *et al.,* (1985)[153] as a facile means of evaluating potential antidepressants. The immobility displayed by rodents when subjected to an unavoidable and inescapable stress has been hypothesized to reflect behavioral despair which in turn may reflect depressive disorders in humans.

Clinically effective antidepressants reduce the immobility that mice display after active and unsuccessful attempts to escape when suspended by the tail.

3.11.1.4 Learned helplessness in rats

Animals exposed to inescapable and unavoidable electric shocks in one situation later fail to escape shock in a different situation when escape is possible.[154] This phenomenon was evaluated as a potential animal model of depression.[155]

3.11.1.5 Muricide behavior in rats

Horovitz *et al.,* (1965) described a selective inhibition of mouse-killing behavior in rats by antidepressants.[156] The test can be used to evaluate antidepressants such as tricyclics and MAO inhibitors.

3.11.1.6 Behavioral changes after neonatal clomipramine treatment

Vogel *et al.,* (1988) reported that neonatally administered clomipramine produces changes in adult rats that resemble endogenous depression in man, based on earlier observations by Mirmiran *et al.,* (1981).

3.11.1.7 Antidepressant-like activity in differential reinforcement of low rate 72-second schedule

The differential-reinforcement of low-rate (DRL) 72-s schedule has been recommended for evaluation of antidepressant drugs.[157]

3.11.1.8 Hole Board test:[158]

The study was conducted using a wooden hole-board apparatus measuring 20 cm by 40 cm with 16 evenly spaced holes (each of diameter 3 cm). The apparatus was elevated to a height of 25 cm. Thirty minutes after treatment, mice were placed singly on the center of the board and the number head dipping and the latency until the first entry was counted using a tally counter during a 5 min trial period

3.11.2 Tests for antidepressant activity based on the mechanism of action

3.11.2.1 Potentiation of norepinephrine toxicity

Antidepressants block the re-uptake of biogenic amines into nervous tissue. In this way, the toxic effects of norepinephrine are potentiated.[159]

3.11.2.2 Compulsive gnawing in mice

In man and in other species, like dogs, apomorphine induces emesis. Treatment of rodents with apomorphine causes compulsive gnawing instead of vomiting. The compulsive gnawing in mice induced by apomorphine is due to dopaminergic stimulation. Centrally acting anticholinergics shift the balance between acetylcholine and dopamine resulting in an enhancement of the apomorphine effect. Therefore, many compounds with psychotropic activity are known to have an apomorphine-synergistic effect. This enhancement is also found after administration of tricyclic antidepressants.[160]

3.11.2.3 Apomorphine-induced hypothermia in mice

Apomorphine induces hypothermia in mice which can be prevented by antidepressants.[161]

3.11.2.4 Tetrabenazine antagonism in mice

Tetrabenazine (TBZ) induces a depletion of biogenic amines (e.g. noradrenaline, dopamine, and serotonin) without affecting their *de-novo* synthesis. TBZ depletes noradrenaline from nerve terminals and prolongs re-uptake into the granula. Noradrenaline is degraded by Monoamine-oxidase. Antidepressants inhibit the re-uptake of noradrenaline into the nerve terminals and increase thereby the noradrenaline concentration at the receptor site. In this way, the effect of TBZ is antagonized. Therefore, both MAO-inhibitors and tricyclic antidepressants are known to prevent or to antagonize these effects. The prevention of TBZ induced ptosis and catalepsy can be used for evaluation of antidepressants.[162]

3.11.2.5 Reserpine-induced hypothermia

Depletion of biogenic amines (noradrenaline, 5-hydroxytryptamine, and dopamine) in the brain induces not only catalepsy and ptosis but also hypothermia in rodents. The decrease in body temperature induced by reserpine is antagonized by antidepressants, MAO- inhibitors, and central stimulants.[163]

3.11.2.6 5-hydroxytryptophan potentiation in mice

According to the monoamine hypothesis of depression, compounds exert antidepressant activity because they are capable of enhancing central noradrenergic and/or serotoninergic functions. Several antidepressant agents potentiate serotonin effects by a block of the re-uptake of serotonin. DL-5-Hydroxytryptophan is used as the precursor of serotonin. The enzymatic breakdown is inhibited by the MAO-inhibitor pargyline. Concurrent administration of 5HTP and MAO inhibitors potentiated 5 HT release result in characteristic head-twitches.[164]

3.11.2.7 5-hydroxytryptophan potentiation in rats

The inhibition of serotonin re-uptake by some antidepressants can be tested *in vivo* in rats by administration of the precursor 5-hydroxytryptophan and inhibition of its breakdown by the MAO-inhibitor pargyline. In contrast to mice exhibiting head twitches, rats show other symptoms such as continuous forelimb clonus.[165]

3.11.2.8 Yohimbine toxicity enhancement

Yohimbine occupies central α_2-receptors and prevents noradrenaline from binding to these receptors. Compounds with antidepressant properties are known to inhibit physiological inactivation of noradrenaline and other biogenic amines by blocking the re-uptake at nerve terminals. Administration of a test compound with antidepressant properties leads to an increase in noradrenaline concentration. Following the simultaneous administration of yohimbine and antidepressants, the animals die of noradrenaline poisonin.[166]

3.11.2.9 Tryptamine seizure potentiation in rats

Monoamine oxidase (MAO) inhibitors like iproniazid enhance seizures in rats caused by an intravenous infusion of tryptamine HCl. This procedure can be used to elucidate the *in vivo* MAO inhibiting properties of compounds.[167]

3.11.2.10 Serotonin syndrome in rats

Compounds which stimulate serotonin receptors cause a series of behavioral changes in rats which is called the serotonin syndrome such as head weaving, increased locomotion, forepaw treading, flat posture and lower lip retraction. With increasing knowledge about the subtypes of serotonin receptors, these symptoms were defined to be associated with 5-HT $_{1A}$ receptors and their specific agonists.[168]

3.11.2.11 Hypermotility in olfactory-bulbectomized rats

Bilateral olfactory bulbectomy in the rat is associated with changes in exploratory behavior that are reversed by chronic, but not acute treatment with antidepressant drugs.[169]

3.11.2.12 Sexual behavior in male rats

Sexual behavior in male rats is stimulated by 5-HT_{1A} receptors agonists[170] and inhibited by serotonin receptor antagonists and by 5-HT_{1B} receptors antagonists.[171]

3.11.3 *In- vitro* method

3.11.3.1 Inhibition of [^{3}H]-norepinephrine uptake in rat brain synaptosomes

As shown by Hertting and Axelrod (1961) the neuronal re-uptake mechanism for norepinephrine is the most important physiological process for removing and inactivating norepinephrine in the synaptic cleft. This uptake is inhibited by cocaine, certain phenylethylamines, and antidepressants. This mechanism is considered as one of the most important modes of action of antidepressants leading to receptor down-regulation. In the brain, the hypothalamus shows the highest level and greatest uptake of noradrenaline. Therefore, this region is used for testing potential antidepressant drugs.

3.11.3.2 Inhibition of [3H]-dopamine uptake in rat striatal synaptosomes

High affinity, saturable, temperature and sodium-dependent transport of 3H-dopamine has been observed in various tissue preparations from different brain regions. The area striata have a high content of dopamine and is suitable for uptake experiments. The 3H-dopamine uptake is inhibited by cocaine, certain phenylethylamines and antidepressants like nomifensine and bupropion but not by tricyclic antidepressants. The test can be used to characterize the mode of action of antidepressant drugs.

3.11.3.3 Inhibition of [3H]-serotonin uptake in synaptosomes

Some authors have suggested that patients with serotoninergic hypofunction constitute a subgroup of depression and claim that altered serotoninergic function determines the mood changes associated with affective disorders. A number of clinically effective antidepressants block the reuptake of 5-HT. 3H-5-HT transport in the brain has been found to be saturable, sodium and temperature-dependent, to be inhibited by several agents such as ouabain, tryptamine analogs and tricyclic antidepressants. Apparently, the 5-HT uptake can be differentiated from catecholamine uptake. Therefore, the test can be used to detect compounds that inhibit serotonin uptake into rat brain synaptosomes and may be potential antidepressants.

PLANT PROFILE:[172]

Botanical name: *Malus domestica*

Family: Rosaceae

SCIENTIFIC CLASSIFICATION

Table 7: Scientific classification of *Malus domestica*

KINGDOM	Plantae
PHYLUM	Magnoleophyta
CLASS	Magnoliopsida
ORDER	Rosales
FAMILY	Rosaceae
GENUS	*Malus*
SPECIES	*Malus domestica*

COMMON NAMES

English: Orchard apple

Hindi: Seb

Sanskrit: Seva

Kannada: Sebu

Malayalam: Appil

Tamil: Apil

Marathi: Sapharacanda

Bengali: Apel

Parts recommended for use as medicine: Fruit, bark, leaves, seeds, flowers and oil.

BOTANICAL DESCRIPTION

DESCRIPTION:

Small and deciduous, reaching 3 to 12 metres (9.8 to 39 ft) tall, with a spreading canopy. Size and shape of the plant is mainly depends on rootstock and training system.

a. Leaves :

Colour : Dark green.

Size : 5-12 cm long and 3-6 cm broad.

Shape : simple oval, arranged alternatively elliptical with serrate margin with an acute tip.

b. Blossoms : Blossoms are produced in spring simultaneously with the budding of the leaves.

c. Flower :

Colour : White in colour with pink tinge that gradually fades and five petaled.

Size :2.5-3.5 cm in diameter.

d. Fruit :

Size : Typically ranges from 5-9 cm in diameter.

Colour :Different in colour, it depends on various species.

The center of the fruit contains five carpels arranged in a five point star, each carpels containing 1-3 seeds.

Figure 24: Photo of *Malus domestica* fruit:

Figure 25: Photo of *Malus domestica* tree:

Distribution:

Malus domestica distributes around 20 countries all over the world and normally in India, Asia, Africa, North & South America, and Europe. In India it is commonly seen in some varieties from Uttaranchal appear during the late summer months, and the Jammu and Kashmir‟s apple season may stretch into late November.

There are several hundred varieties of apples, grown all over the world. Apples trees grow best in temperate countries with a cool climate and plenty of rain during winter. The apple has been an important food plant in Europe and Asia for thousands of years. The continents of Europe and North America are the main sources of supply, but apples are also produced in Australia, New Zealand, South Africa, and some part of Asia.[173]

Apple varieties, of which there are thousands, fall into three broad classes: (1) cider varieties; (2) cooking varieties; and (3) dessert varieties, which differ widely but tend to emphasize color, size, aroma, smoothness, and perhaps crispness and tang. Many varieties are relatively high in sugar, only mildly acidic, and very low in tannin. Apples provide vitamins A and C, are high in carbohydrates, and are an excellent source of dietary fiber.

CHEMICAL CONSTITUENTS:

Apple contains a large concentration of flavonoids, as well as a variety of other phytochemicals, and the concentration of these phytochemicals may depend on many factors, such as cultivar of the apple, harvest and storage of the apples, and processing of the apples. Concentration of phytochemicals also varies greatly between the apple peels and the apple flesh.[174] Apples are freshly fruit with high water content about (85%) and a low sugar content (on average, about 10-12% weight). The main carbohydrates are fructose (6%), glucose (2.2%).

The most well studied antioxidant compounds in the apples are quercetin-3 galactoside, quercetin-3-glucoside, quercetin-3-rhamnoside, catechin, epicatechin, procyanidin, cyanidin-3-galactoside, coumaric acid, chlorogenic acid, gallic acid, and phloridzin.

Recently researchers have examined the average concentrations of the major phenolic compounds in six cultivars of apples. They found that the average phenolic concentrations among the six cultivars were: quercetin glycosides, 13.2 mg/100 g fruit; vitamin C, 12.8 mg/100 g fruit; procyanidin B, 9.35 mg/100 g fruit; chlorogenic acid, 9.02 mg/100 g fruit; epicatechin, 8.65 mg/100 g fruit; and phloretin glycosides, 5.59 mg/100 g fruit.[175]

The compounds most commonly found in apple peels consist of the procyanidins, catechin, epicatechin, chlorogenic acid, phloridzin, and the quercetin conjugates. In the apple flesh, there is some catechin, procyanidin, epicatechin, and phloridzin, but these compounds are found in much lower concentrations than in the peels. Chlorogenic acid tends to be higher in the flesh than in the peel.[176]

Apple peels contain higher antioxidant compound especially quercetin, it has higher antioxidant activity and higher bioavailability then the apple flesh. Apples with the peels were also better able to inhibit cancer cell proliferation when compared to apples without the peels 65. More recent work has shown that apple peels contain two to six times (depending on the variety) more phenolic compounds than in the flesh, and two to three times more flavonoids in the peels when compared to the flesh.[177]

THERAPEUTIC USES:

"An apple a day keeps doctor away". *Malus domestica* (Apple) a traditional plant widely used since Iron Age and has multiple benefits. Apart from pharmacological screening of *Malus domestica* edible properties of apples reportedly used for natural therapies as follows.[178]

ANTACID:

It makes it very suitable to stop stomach acidity due to it contains pectin as well as the influence of the glycine.

ANTI-DIARRHEAL AND SOFT LAXATIVE:

An apple seems contradictory its high content in pectins turn it into a good regulator of intestinal tract and absorbent value of the pectins make ideal case of colitis, diarrhea, gastroenteritis and in all those cases in that a too abundant and soft defecation is produced.

DIURECTIC AND DEPURATIVE:

Apple content such as cysteine and arginine as well as the malic acid, it is very appropriate to eliminate the toxins that are stored in the body and besides fighting or preventing the diseases. It recommended in affection like uric acid, gout, urticarial and for the treatment of kidney related diseases.

- **HEARING LOSS:**

An apple vinegar has very beneficial properties to the health of the ear because it rich in potassium, magnesium zinc and manganese. One of this mineral deficiency can cause deafness.

PHARMACOLOGICAL ACTIVITIES- AN EVIDENCE BASED APPROACH

1. ANT-OXIDANT ACTIVITY:

Apple, and especially its peels have been found to have a potent antioxidant activity and can greatly inhibit the growth liver cancer and colon cancer cells. The total antioxidant activity of apples with the peel was approximately 83μmol vitamin-C equivalents, which means that the antioxidant activity of 100 g apples is equivalent to about 1500 mg of vitamin-C. However, the amount of vitamin-C in 100 g of apples is only about 5.7 mg. Vitamin-C is a powerful antioxidant, but this research shows that nearly all of the antioxidant activity from apples comes from a variety of other compounds. Vitamin-C in apples contributed less than 4% of total antioxidant activity.[179]

2. ANTI-PROLIFERATIVE ACTIVITY:

Apples have been shown to have potent antiproliferative activity in several studies. When Caco-2 colon cancer cells were treated with apple extracts, cell proliferation was inhibited in a dose-dependent manner reaching a maximum inhibition of 43% at a dose of 50 mg/mL. The same trend was seen in Hep G2 liver cancer cells with maximal inhibition reaching 57% at a dose of 50 mg/mL. Due to its unique combination of phytochemicals in the apples that are responsible for inhibiting the growth of tumor cells.[180] Apples had the third highest antiproliferative activity when compared to eleven other commonly consumed fruits. Apples without peels were significantly less effective in inhibiting Hep G2 cell proliferation when compared to apples with the peel, suggesting that apple peels possess significant antiproliferative activity. It was concluded that apple peels alone inhibited Hep G2 cell proliferation significantly more than whole apples.[181]

3. ANTI-MICROBIAL ACTIVITY:

Effect of water and alcohol extracts of *M.domestica* fruit was found to be most effective against gram +ve and gram –ve bacteria such as B.subtilis, S.aureus and E.coli, P.aeuroginosa respectively.[182]According to literature, the antimicrobial activity could be influenced by the phenolic compounds and their polyphenol extracts had stronger inhibition effects on the bacteria. An *in vivo* assay is necessary to confirm the antimicrobial activities of *Malus domestica*, which could be usefully applied to the food, pharmaceuticals, and cosmetics industries. Isolation of the gene responsible for the antimicrobial activity would be an interesting future study topic aimed at identifying the molecule generating the desirable efficacy.

4. INHIBITION OF LIPID OXIDATION:

It has been found that addition of apple phenolics to human serum decreased diphenylhexatriene- labeledphosphatidylcholine (DPHPC) oxidation in a dose dependent manner. DPHPC is incorporated into low-density lipoprotein (LDL), high-density lipoprotein and very low- density lipoprotein (VLDL) fractions and is an indicator of oxidation. Apple ingestion led to a decrease in DPHPC oxidation, reflecting the apples antioxidant activity in vivo. The protective effects of apples on LDL oxidation reached its peak at three hours following apple consumption and returned to baseline levels by 24 hours.

Diphenylhexatrienelabeled propionic acid (DPHPA) binds to serum albumin and is a good measure of oxidation within the aqueous phase of human serum. It was also found that consumption of apples also led to a decrease in albumin DPHPA oxidation, reaching peak activity at 3 hours.[183]

5. ANTI-DEPRESSANT ACTIVITY:

Apple ripe with antioxidants and fiber. An apple a day could if eaten with the rest of these foods keep the psychiatrist away, at least for stretches of time. Like berries, apples are high in antioxidants, which can help to prevent and repair oxidation damage and inflammation on the cellular level. Apple juice consumption may increase the production in the brain of the essential neurotransmitter acetylcholine. The researchers found that including apples in your daily diet may protect neuron cells against oxidative stress-induced neurotoxicity.[184]

6. CHOLESTEROL LOWERING EFFECT:

Effect of *M.domestica* supplementation serum lipids and lipoproteins level in cholesterol-fed male rat showed that supplementation of *M.domestica* reduced the amount of TC, LDL and TG and increased HDL concentration. The effect can be due to antioxidant effect of compounds constituting the food was linked probably by inhibiting lipid peroxidation and decrease production of cholesterol, LDL and triglycerides.[185]

7. ANTI-INFLAMMATORY EFFECT:

Effect of anti-inflammatory action of apple polyphenol extracts prevents damage to human gastric epithelial cells in vitro and to rat gastric mucosa in vivo. It was concluded that apple extract reduces gastric erosion in indomethacin(at dose of 35 mg/kg, s.c) induced injury in rats because of Phenolic compounds have been shown to exert direct antioxidant effects acting as ROS scavengers, hydrogen donating compounds, singlet oxygen quenchers, and metal ion chelators.[186]

8. ASTHMA AND PULMONARY FUNCTION:

Apple consumption has been inversely linked with asthma and has also been positively associated with general pulmonary health. It has been found that an apple and pear intake was associated with decreased risk of asthma and a decrease in bronchial hypersensitivity because of it contains high concentration of antioxidant, vitamins, phenolic acid and flavonoids which helping to calm the inflammation in the airway.[187]

9. DIABETES AND WEIGHT LOSS:

An apple consumption may also be associated with a lower risk for diabetes and its peels was also associated with a decreased risk in type II diabetes due to apple contains in higher concentration of quercetin.

A study was conducted on approximately 400 hypercholestemic but nonsmoking women were randomized to one of three supplement groups: oat cookies, apples or pears, and each subject consumed one of each supplement three times per day for twelve weeks. The participants who consumed either of the fruits had a significant weight loss after 12 weeks of 1.21 kg, whereas those consuming the oat cookies did not have a significant weight loss. Those consuming fruit also had a significantly lower blood glucose level when compared to those consuming the oat cookies.[188]

OTHER HEALTH EFFECTS:

Recently it has been found that crude extracts from immature apples actually inhibited enzymatic activities of cholera toxin in a dose dependent manner. Additionally, apple extracts reduced cholera toxin induced fluid accumulation in a dose dependent manner. The apple extracts were fractionated and each fraction was tested for inhibitory action on enzymatic activities of cholera toxin. The two apple extract fractions that contained highly polymerized catechins inhibited cholera toxin catalyzed ADP-ribosylation by 95% and 98%. The fraction containing non-catechin polyphenols caused only 3.5% inhibition and the fraction containing monomeric, dimeric, and trimericcatechins caused 39% inhibition.[189]

Chapter 4

Methodology

METHODOLOGY

4.1 MATERIALS AND METHODS

Table 8: List of materials used during experiment

Sl. No.	Chemicals	Supplied by
1.	Tween 80-1%	Medlife
2.	Imipramine	Yarrow chem products
3.	Sucrose	S.D. Fine Chemicals Ltd
4.	Tris	Hi Media Laboratories
5.	EDTA disodium salt	Hi Media Laboratories
6.	Sodiun dihydrogen phosphate	Hi Media Laboratories
7.	Disodium hydrogen phosphate	Hi Media Laboratories
8.	Serotonin hydrochloride	Merck India Ltd.
Instruments used		
1.	Animal weighing electronic balance	Scale-Tec, Model- CTG 600
2.	Chemical weighing balance	VAMAN, VA30, made in Mumbai
3.	UV/visible spectrophotometer	Model: Jasco V-530, Japan
4.	Heating water bath	Remi Scientific labs Mumbai
5.	Refrigerated centrifuge	Lab enterprises, India
6.	Hole board	
7.	Juicer Blender	Wonderchef nutri-blend ultima

4.2 Apparatus

Quartz cuvette, centrifuging tubes (Tarson, India), Glass beaker, test tubes, tissue paper, volumetric flasks and micropipettes were purchased from authorized vendors.

4.3 CHEMICALS AND INSTRUMENTS

All chemicals and solvents used in the study were of analytical grade were used for the present study.

4.4 COLLECTION AND AUTHENTICATION OF PLANT MATERIAL:

The fresh *Malus domestica* fruits used for the present studies were procured from local market of Gurpura Kaikamba , Mangalore, Dakshina Kannada district Karnataka, in Dec 2018. It was authenticated by Dr. Jyothi Miranda, Associate Professor, Department of Botany, St. Aloysious College (Autonomous), Mangalore.

4.5 PREPARATION OF FRUIT JUICE:

The method of juicing includes, weighing the fresh fruit, (80gm) cut into appropriate sizes and mixed using a blender for two minutes. The pulp obtained was squeezed using muslin cloth and the juice (15ml-20ml/100g) is refrigerated and used for the anti-depressant studies.

Figure 26: Preparation of fruit juice

Malus domestica

Apple juice blender

Fresh fruit juice of *Malus domestica*

4.6 PRELIMINARY QUALITATIVE PHYTOCHEMICAL ANALYSIS[190]:

A. Detection of alkaloids:

Fresh fruit juice extract was tested for presence of alkaloids as follows:

Table 9: Preliminary Phytochemical analysis

Sl. No	Test	Observation	Inference
1.	**Mayer's test:** To few ml of extract, 2 drops Mayer‟s reagent was added along sides of tube.	Formation of creamy precipitate	Presence of Alkaloids
2.	**Dragendroff's test:** 1ml of Dragendroff‟s reagent was added to few ml of extract.	Formation of orange or orange-red precipitate	Presence of Alkaloids
3	**Wagner's test:** To few ml of extract, few drops of Wagner‟s reagent were added along sides of tube.	Formation of reddish brown precipitate	Presence of Alkaloids
4	**Hager's test**: Hager‟s reagent was added to few ml of the extract.	Formation of yellow precipitate	Presence of Alkaloids

B. Detection of carbohydrates:

Fresh fruit juice extract was tested for presence of Carbohydrates and the extract was subjected to the following tests

Sl. No	Test	Observation	Inference
1	**Molisch's test**: To 2ml of the extract, 2 drops of alcoholic solution of alpha naphthol were added. The mixture was shaken well and 1ml conc. H_2SO_4 was added along the sides of the tube, and allowed to stand.	Formation of violet ring at the junction of layers	Presence of Carbohydrates
2	**Fehling's test**: To 1ml extract, 1mL each of Fehling‟s solutions A & B were added & heated on water bath.	Formation of red precipitate	Presence of Carbohydrates
3	**Barfoed's test**: 1ml Barfoed‟s reagent was added to 1mL of extract and heated for 2 minute.	Formation of red precipitate	Presence of Carbohydrates
4	**Benedict's test**: 0.5mL of the extract was added to 0.5mL of Benedict‟s reagent and heated on water bath for 2 min.	Formation of brick red ppt.	Presence of Carbohydrates
5	**Seliwanoff's test:** Extract and Seliwanoff‟s reagent were mixed and heated strongly.	Formation of cherry red color	Presence of Carbohydrates

C. Detection of flavonoids:

Sl. No	Test	Observation	Inference
1	**Shinoda test:** A little quantity of extract was dissolved in alcohol + few fragments of Mg turnings + conc. HCl drop wise.	Presence of pink or crimson-red color	Presence of flavonoids
2	**Lead acetate test**: Lead acetate solution was added to small amount of extract.	Formation of yellow precipitate	Presence of flavonoids
3	**Alkaline reagent test:** Increasing amount of sodium hydroxide was added to the sample of extract.	Formation of yellow coloration observed which disappears upon addition of acid	Presence of flavonoids
4	**Ferric chloride test:** Extract + Ferric chloride solution.	Formation of green to black color	Presence of flavonoids.

D. Detection of Glycosides:

Sl. No	Test	Observation	Inference
1	**Baljet test:** Test extract was treated with sodium pirate.	Formation of yellow to orange colour.	Presence of glycosides
2	**Keller-Killiani test:** The test extract was treated with few drops of ferric chloride solution and mixed. Then, conc. Sulphuric acid containing ferric chloride solution was added.	Formation of two layers, lower layer reddish brown and upper acetic acid layer with bluish green colour.	Presence of glycosides
3	**Bromine water test:** Test extract was dissolved in bromine water.	Formation of yellow precipitate.	Presence of glycosides
4	**Legal's test:** Test extract was treated with pyridine (made alkaline by adding sodium nitroprusside solution)	Formation of pink to red colour.	Presence of glycosides

E. Detection of Saponins:

Sl. No.	Test	Observation	Inference
1	**Froth's test:** The extract was diluted with distilled water and shaken for 15 min.	Foam formation	Presence of Saponins
2	**Sulphur test:** Sulphur was added to the sample of extract.	Characteristic observation	Presence of Saponins

F. Detection of Steroids:

Sl No.	Test	Observation	Inference
1	**Lieberman Burchard's test**: 2mg of dry extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1mL of conc. H_2SO_4 added.	No Formation of red violet or green color.	Absence of steroids
2	**Salkowski reaction**: 2mg of dry extract was shaken with $CHCl_3$. To the $CHCl_3$ layer, H_2SO_4 was added slowly along the sides of test tube.	No Formation of red color	Absence of steroids

I. Detection of Inositol:

G. Detection of Tannins:

Sl No.	Test	Observation	Inference
1	**Ferric chloride test**: Few drops of 5% w/v $FeCl_3$ solution was added to 1-2ml of the extract.	Formation of brown color.	Presence of tannins
2	**Vanillin hydrochloride test:** Extract was treated with vanillin hydrochloride Reagent	Formation of purplish red color.	Presence of tannins
3	**Gelatin test:** Extract was treated with gelatin solution.	Formation of white precipitate	Presence of Tannins

H. Detection of Proteins:

Fresh fruit juice extract was tested for presence of Proteins and the extract was subjected to the following tests

Sl No.	Test	Observation	Inference
1	**Millon's test:** 2ml of the extract was added to few drops of Millon‟s reagent.	Formation of white precipitate	Presence of protein
2	**Biuret test:** To 2ml of extract + 1 drop of 2% $CuSO_4$ + 1 ml of 95% ethanol + excess of KOH pellets were added	Formation of pink color in the ethanolic layer	Presence of protein
3	**Ninhydrin test:** 2 drops of ninhydrin solution was added to 2ml of extract.	Formation of purple colour	Presence of amino acid

Sl No.	Test	Observation	Inference
1	Sample + nitric acid, evaporate: contains acidic keto compounds. Then add calcium chloride + ammonia and evaporate	Formation of red colour	Presence of inositol

4.7 Storage conditions

The test compounds and its suspensions were prepared freshly on the day of experiment and stored in airtight amber colored vials to protect from exposure to sunlight during the experiments.

4.8 Volume of test compounds

The volume of test compounds was calculated based upon the body weight of animal.

4.9 Route of administration

The test compounds were administered per orally.

4.10 EXPERIMENTAL ANIMALS:

Healthy Swiss albino mice (22-25g) of either sex were used for the experiment were procured from the animal house of Srinivas College of Pharmacy, Mangalore. They were maintained under standard conditions (temperature 22 ± 2^{o}C, relative humidity 60±5% and 12 hours light/dark cycle). The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellet diet and water *ad libitum*.

4.11 APPROVAL OF RESEARCH PROTOCOL

The Institutional Animal Ethics Committee approved the experimental protocol (Approval no SCP/IAEC/F150/P122/2017 dated 28.11.2017). All the animals received human care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the "National Academy of Sciences" and published by the "National Institute of Health". The animals were acclimatized for at least one week before use.

4.12 EVALUATION OF ANTI DEPRESSANT ACTIVITY

4.12.1 PHARMACOLOGICAL EVALUATION:

PREPARATION OF DOSE:

The fruit juice of the *Malus domestica* fruit was prepared at a dose of 1000 mg/kg body weight of animal and administered 0.5ml/100g body weight of the animal.

Figure 27: Oral route administration of drugs in mice

4.12.2 EVALUATION OF ANTIDEPRESSANT ACTIVITY

A. Forced Swim Test (FST)

Swiss albino mice (20-25 g) were divided into following group containing 6 mice in each group.

Treatment groups:

Group 1 : Vehicle control (water , p.o.)

Group 2 : Imipramine (10 mg/kg, p.o.)

Group 3 : FFJMD low dose (0.5 ml, p.o.)

Group 4 : FFJMD high dose (1 ml, p.o.) respectively

Mobile **Immobile**

Figure 28: Forced swim test in mice (mobile- immobile)

Experimental design:

Mice were divided into 4 groups. Each group contains six animals. The group 1 treated as vehicle control, Group 2 received standard drug imipramine (10 mg/kg p. o.) and group 3, 4 were treated with FFJMD (0.5ml and 1ml) respectively. The treatment was given once daily, continuously for 10 day. On the 9^{th} day 60 min after the regular drug treatment, individual mouse was subjected to forced swim test as described by Porsolt *et al* (1979)[191]. A mouse was individually placed in vertical plexiglass cylinder (Height: 38cm; Width 75 cm) containing water maintained at 26^0 $\pm 1^0$C. Two swimming sessions were conducted as pre-test session (15 min habituation) and 24 h later the test session (6 min).

During pre-test session mouse was allowed to swim for 15 min and removed thereafter and dried for 15 min in a heated enclosure (32^0 C) and then returned to home cages. Water in the cylinder was changed after subjecting each animal to FST because used water has been shown to alter the behavior.[192] The treatment was given before test session on the 10^{th} day i.e. after 24 hr the test session was commenced by placing individual mouse in cylinder with same condition as in pre-test session. The duration of immobility was recorded during the next 4 min of the total 6 min testing period.

Mice were judged to be immobile whenever they ceased struggling and remained floating passively in the water in a slightly hunched but upright position, its head just above the surface.

At the end of the study on 10^{th} day animals were sacrificed by cervical dislocation. Brains were removed and cleaned and immediately homogenized for the estimation of activity of monoamine oxidase enzyme.

B. BIOCHEMICAL ESTIMATION:
Measurement of brain MAO-A activity[193]

At the end of the experiment, mice were sacrificed and the brain samples were collected and mouse brain mitochondrial fractions were prepared following the procedure of Schurr and Livne, (1976). The MAO activity was assessed spectrophotometrically. Briefly, the buffer, washed brain sample was homogenized in 9 volumes of cold 0.25 M sucrose, 0.1 M Tris, 0.02 M EDTA buffer (pH 7.4) and centrifuged twice at 800 g for 10 min at 4°C in cooling centrifuge (Remi instruments, Mumbai, India). The pellet was discarded. The supernatant was then centrifuged at 12000 g for 20 min at 4°C in cooling centrifuge. The precipitates were washed twice with about 100 ml of sucrose-Tris-EDTA buffer and suspended in 9 volumes of cold sodium phosphate buffer (10 mM, pH 7.4, containing 320 mM sucrose) and mixed well at 4°C for 20 min. The mixture was then centrifuged at 15000 g for 30 min at 0°C and the pellets were re-suspended in cold sodium phosphate buffer. The protein concentration was estimated by Lowry method, (1951) using bovine serum albumin as the standard.

For estimating MAO A activity, 2.75 ml sodium phosphate buffer (100 mM, pH 7.4) and 100 µl of 4 mM 5-hydroxytryatpamine were mixed in a quartz cuvette. This was followed by the addition of 150 µl solutions of the mitochondrial fraction to initiate the enzymatic reaction and the change in absorbance was recorded by a double beam spectrophotometer (JASCO, Japan) at a wavelength of 280 nm against the blank containing sodium phosphate buffer (100 mM) and 5 hydroxytryptamine (4 mM).

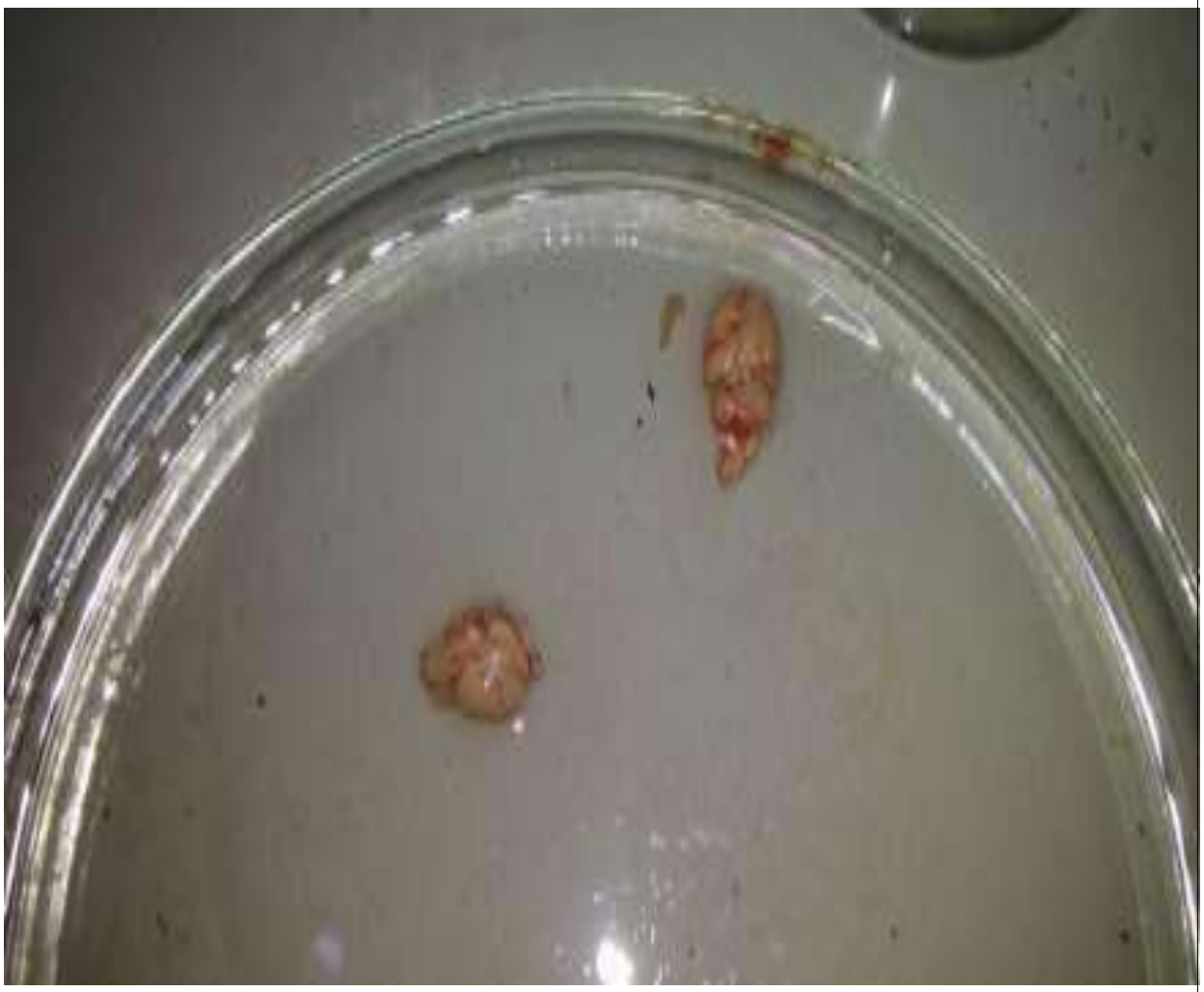


Figure 29: The brain extracted for the estimation of biochemical parameters

C. Tail suspension test:[194]

Swiss albino mice (18-22g) were used for the study and divided into eight groups of 6 mice per group.

Treatment groups:

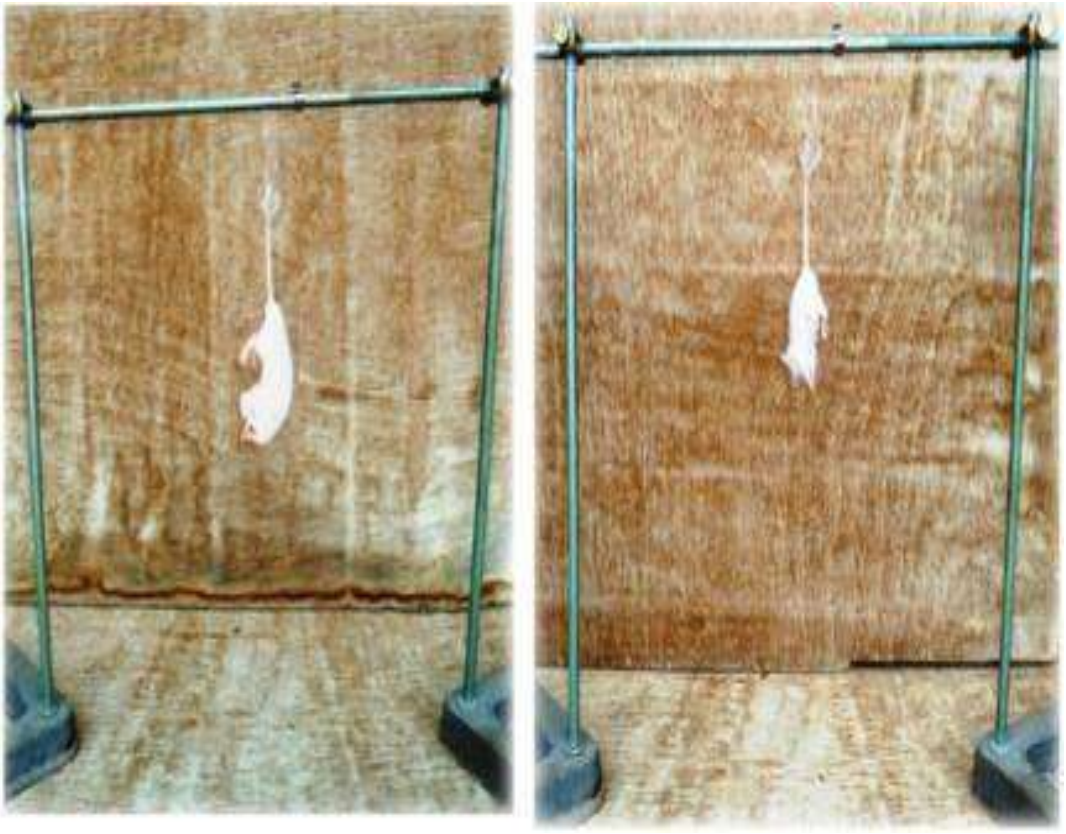
Group 1 : Vehicle control (water , p.o.)

Group 2 : Imipramine (10 mg/kg, p.o.)

Group 3 : FFJMD low dose (0.5 ml, p.o.)

Group 4 : FFJMD high dose (1 ml, p.o.) respectively

Figure 30: Tail suspension test in mice (mobile- immobile)



Mobile **Immobile**

Experimental design:

The tail suspension test was conducted in brief, mice were divided into 4 groups each group contain six animals. The group 1 treated as vehicle control, Group 2 received standard drug imipramine (10 mg/kg p. o.) and group 3, 4 were treated with FFJMD (0.5ml and 1ml) respectively. The treatment was given once daily, continuously for 10 days. On the 10^{th} day 60 min after the regular drug treatment, individual mouse was suspended on the suspending rod, 58 cm above a table top by adhesive tape placed approximately 2 cm from the tip of the tail. The head of mouse was 50 cm away from the nearest object and was both acoustically and visually isolated. The duration of immobility was recorded for a period of 6 min. Mice was considered immobile when they remain passively and completely motionless for at least 1 min.

D. Hole board test:

Swiss albino mice (18-22g) were used for the study and divided into eight groups of 6 mice per group.

Treatment groups:

Group 1 : Vehicle control (water , p.o.)

Group 2 : Imipramine (10 mg/kg, p.o.)

Group 3 : FFJMD low dose (0.5 ml, p.o.)

Group 4 : FFJMD high dose (1 ml, p.o.) respectively

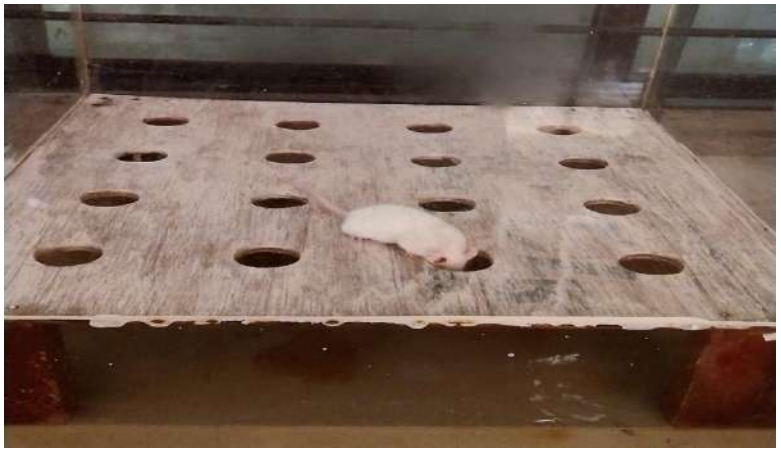


Figure 31: Hole board test in mice

Experimental design:[195]

The hole board test was conducted as described earlier by. In brief, mice were divided into 4 groups each group contain six animals. The group 1 treated as vehicle control, Group 2 received standard drug imipramine (10 mg/kg p. o.) and group 3, 4 were treated with FFJMD (0.5ml and 1ml) respectively. The treatment was given once daily, continuously for 10 days. On the 10^{th} day 60 min after the regular drug treatment, individual mouse was placed in the hole board apparatus. The study was conducted using a wooden hole-board apparatus measuring 20 cm by 40 cm with 16 evenly spaced holes (each of diameter 3 cm). The apparatus was elevated to a height of 25 cm. Thirty minutes after treatment, mice were placed singly on the center of the board and the number head dipping and the latency until the first entry was counted using a tally counter during a 5 min trial period.

4.12.3 STATISTICAL ANALYSIS

The data of pharmacological experiments were expressed as mean ± standard error mean (SEM). Data analysis was performed using Graph Pad Prism 5.0 software (Graph Pad, San Diego, CA, USA). Data of 5-HTP induced head-twitch responses in mice was analyzed by two-way analysis of variance (ANOVA) followed by Turkey‟s (multiple comparison test). Data of biochemical parameters were analyzed using one way analysis of variance (ANOVA) followed by Dunnett‟s test. A value of $P<0.05$ was considered to be statistically significant.

Chapter 5

Results

5.1 PRELIMINARY PHYTOCHEMICAL SCREENING:

Results of the preliminary phytochemical investigation of fresh fruit juice of *Malus domestica* is shown in table 10.

Table 10: Preliminary phytochemical screening of FFJMD

Sl. No.	Test	Result
1.	Alkaloids	+ve
2.	Carbohydrates	+ve
3.	Flavonoids	+ve
4.	Glycosides	+ve
5.	Saponins	+ve
6.	Steroids	-ve
7.	Tannins	+ve
8.	Proteins	+ve
9.	Inositol	+ve

(+ve = present in test, -ve = absent in test)

5.2 PHARMACOLOGICAL ASSESSMENT OF ANTIDEPRESSANT ACTIVITY:

A. FORCED SWIM TEST:

In the acute and chronic forced swim test duration of immobility was significantly reduced in the imipramine treated and FFJMD 0.5ml and 1 ml treated groups. There was more significant decrease in immobility in the chronic FFJMD administration (Table 11).

Table 11: Effect of imipramine and FFJMD on immobility period of mice in forced swim tests

Groups	Acute forced swim test immobility in seconds (Mean ± SD)	Chronic forced swim test immobility in seconds (Mean ± SD)
Depressive control	126.8 ± 0.60	127.8 ± 0.94
Standard Imipramine 10 mg/kg	70.17 ± 0.30***	76.83 ± 0.60***
FFJMD 0.5 ml	123.5 ± 0.76**	124.7 ± 1.14*
FFJMD 1 ml	91.83 ± 0.47***	96 ± 0.44***

All the results are expressed in term of mean±SEM n=6 animals in each group; Statistical significance was determined by ANOVA followed by Tukey's test. *P<0.05, **P< 0.01, ***P< 0.001, statistically significant compared to depressive control group.

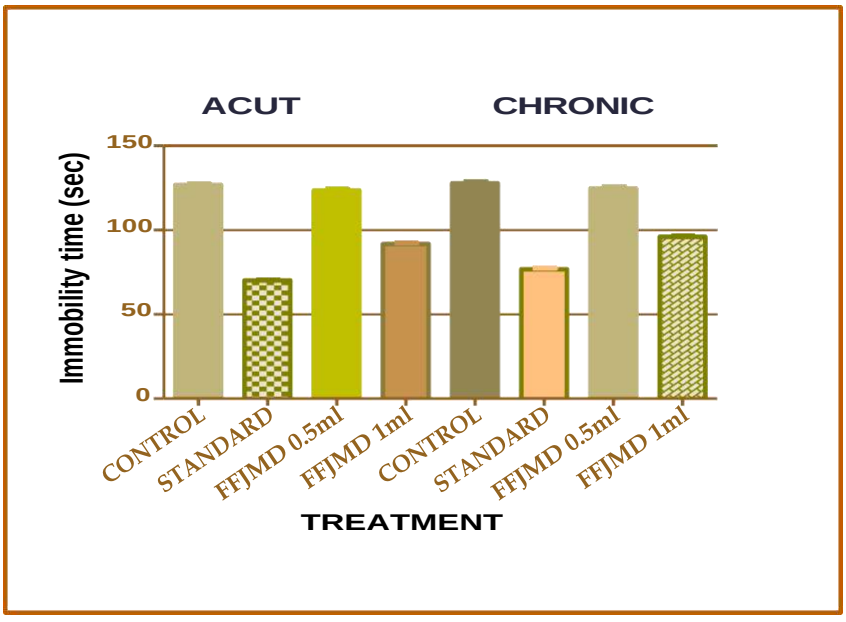


Figure 32: Effect of imipramine and FFJMD on immobility period of mice in acute and chronic forced swim tests

A. MONO AMINO OXIDASE:

The estimation of MAO level which is assessed UV- Visible Spectrophotometer and absorbance recorded at wavelength of 280nm.

Table 12: Effect of *Malus domestica* fruit juice in estimation of mono amino oxidase level in the brain of mice after forced swim tests.

Groups	Treatment	MAO-A activity (nmol/mg protein)	MAO-A activity % Inhibition
1.	Vehicle control	23.67±1.282	
2.	FFJMD 0.5ml	17.50±1.749*	36.52
3.	FFJMD 1ml	15.60±1.806**	48.84

All the results are expressed in term of mean±SEM n=6 animals in each group; Statistical significance was determined by ANOVA followed by Tukey"s test. *P<0.05, **P< 0.01, ***P< 0.001, statistically significant compared to depressive control group.

CALCULATION:

% inhibition = 100 x [1 - (X - MIN) / (MAX - MIN)]

Max- Signal with no inhibition
Min – Signal with 100% inhibition
X- Signal at a given concentration of inhibitor

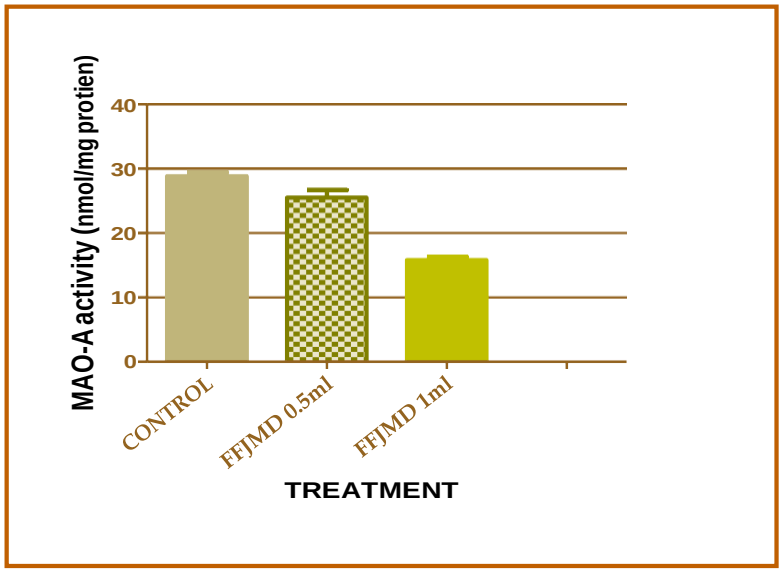


Figure 33: Effect of *Malus domestica* fruit juice in estimation of mono amino oxidase level in the brain of mice

Measurement of brain MAO-A activity

At the end of the experiment, mice were sacrificed and the brain samples were collected and mouse brain mitochondrial fractions were prepared following the procedure of Schurr and Livne, (1976).

The MAO activity was assessed spectrophotometrically. Briefly, the buffer, washed brain sample was homogenized in 9 volumes of cold 0.25 M sucrose, 0.1 M Tris, 0.02 M EDTA buffer (pH 7.4) and centrifuged twice at 800 g for 10 min at 4°C in cooling centrifuge (Remi instruments, Mumbai, India).The pellet was discarded. The supernatant was then centrifuged at 12000 g for 20 min at 4°C in cooling centrifuge. The precipitates were washed twice with about 100 ml of sucrose-Tris-EDTA buffer and suspended in 9 volumes of cold sodium phosphate buffer (10 mM, pH 7.4, containing 320 mM sucrose) and mixed well at 4°C for 20 min. The mixture was then centrifuged at 15000 g for 30 min at 0°C and the pellets were re-suspended in cold sodium phosphate buffer. The protein concentration was estimated by Lowry method, (1951) using bovine serum albumin as the standard. For estimating MAO A activity, 2.75 ml sodium phosphate buffer (100 mM, pH 7.4) and 100 µl of 4 mM 5-hydroxytryatpamine were mixed in a quartz cuvette. This was followed by the addition of 150 µl solutions of the mitochondrial fraction to initiate the enzymatic reaction and the change in absorbance was recorded by a double beam spectrophotometer (JASCO, Japan) at a wavelength of 280 nm against the blank containing sodium phosphate buffer (100 mM) and 5 hydroxytryptamine (4 mM).

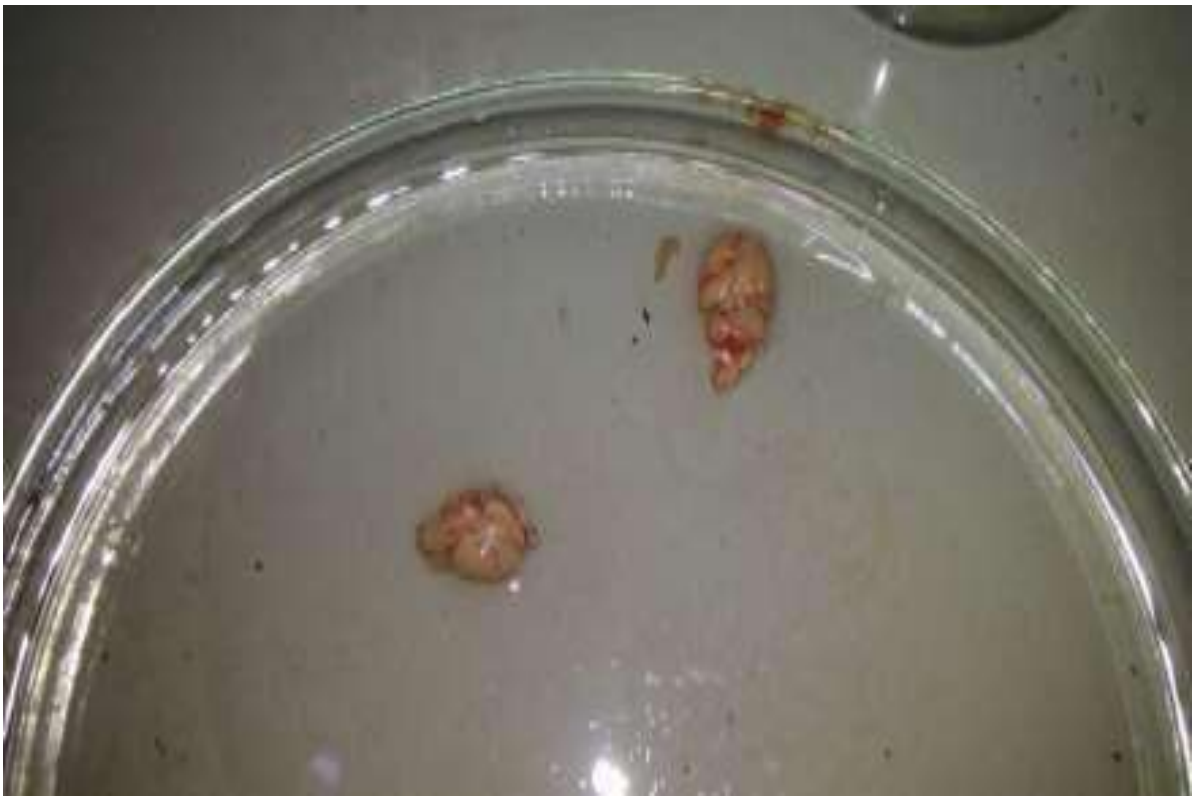


Figure 34: The brain extracted for the estimation of biochemical parameters

C. TAIL SUSPENSION TEST:

In the acute and chronic tail suspension test duration of immobility was significantly reduced in the imipramine treated and FFJMD 0.5ml and 1 ml treated groups. There was more significant decrease in immobility in the chronic FFJMD administration.

Table 13: Effect of imipramine and FFJMD on immobility period of mice in tail suspension tests

Groups	Acute tail suspension test immobility in seconds (Mean ± SD)	Chronic tail suspension test immobility in seconds (Mean ± SD)
Depressive Control	272.8 ± 0.60	289.8 ± 0.94
Standard Imipramine 10 mg/kg	207.2 ± 0.30***	205.8 ± 0.60***
FFJMD 0.5 ml	270.5 ± 0.76*	293.5 ± 0.84**
FFJMD 1 ml	222.8 ± 0.47***	236.0 ± 0.44***

All the results are expressed in term of mean±SEM n=6 animals in each group; Statistical significance was determined by ANOVA followed by Tukey's test. *P<0.05, **P< 0.01, ***P< 0.001, statistically significant compared to depressive control group.

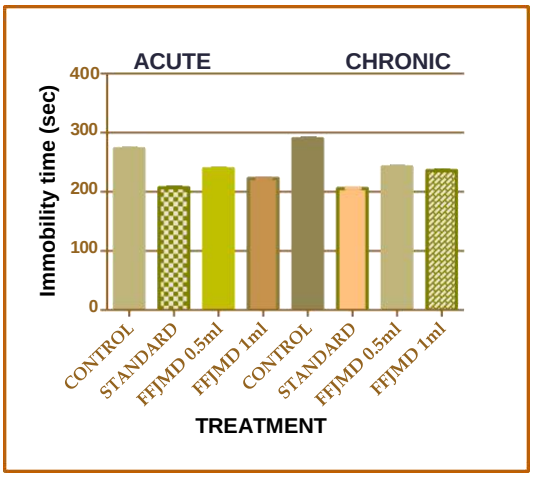


Figure 35: Effect of imipramine and FFJMD on immobility period of mice in acute and chronic tail suspension tests

D. HOLE BOARD TEST:

The data on entries into a new area were used to calculate the total amount of locomotion (number of entries into all areas summed together) and the percentage of entries that were in made into the central area. The location of the animal during each of the 10-s time intervals was used to estimate the percentage of time spent in the central area.

Table 14: Effect of imipramine and FFJMD in mice in acute hole board test

GROUPS	TOTAL LOCOMOTION	% ENTRIES INTO THE CENTRE	% TIME IN THE CENTRE	FREQUENCY OF HEAD DIPPING	FREQUENCY OF REARING
Depressive Control	47.83 ± 0.60	3.83 ± 0.60	2.83 ± 0.60	10.67 ± 0.71	18.83 ± 0.60
Standard Imipramine 10 mg/kg	60.33 ± 0.66***	15.17 ± 0.30***	9.16 ± 0.30***	6.50 ± 0.76***	24.17 ± 0.30***
FFJMD 0.5 ml	41.00 ± 0.36**	6.33 ± 0.42**	5.50 ± 0.42**	2.50 ± 0.76*	16.50 ± 0.42**
FFJMD 1 ml	51.00 ± 0.44***	11.83 ± 0.47***	7.83 ± 0.47***	4.66 ± 0.80***	20.83 ± 0.47***

All the results are expressed in term of mean±SEM n=6 animals in each group; Statistical significance was determined by ANOVA followed by Tukey's test. *P<0.05, **P< 0.01, ***P< 0.001, statistically significant compared to depressive control group.

Table 15: Effect of imipramine and FFJMD in mice in chronic hole board test

GROUPS	TOTAL LOCOMOTION	% ENTRIES INTO THE CENTRE	% TIME IN THE CENTRE	FREQUENCY OF HEAD DIPPING	FREQUENCY OF REARING
Depressive Control	56.50 ± 0.76	10.50 ± 0.76	6.50 ± 0.76	11.00 ± 0.63	24.17 ± 0.87
Standard Imipramine 10 mg/kg	60.83 ± 0.60***	18.83 ± 0.60***	13.83 ± 0.60***	18.83 ± 0.60***	29.83 ± 0.60***
FFJMD 0.5 ml	45.33 ± 0.42*	11.33 ± 0.55**	9.50 ± 0.42**	10.50 ± 0.42*	20.33 ± 0.42**
FFJMD 1 ml	51.50 ± 0.76***	13.00 ± 0.44***	11.00 ± 0.44***	13.67 ± 0.49***	26.00 ± 0.44***

All the results are expressed in term of mean±SEM n=6 animals in each group; Statistical significance was determined by ANOVA followed by Tukey‟s test. *P<0.05, **P< 0.01, ***P< 0.001, statistically significant compared to depressive control group.

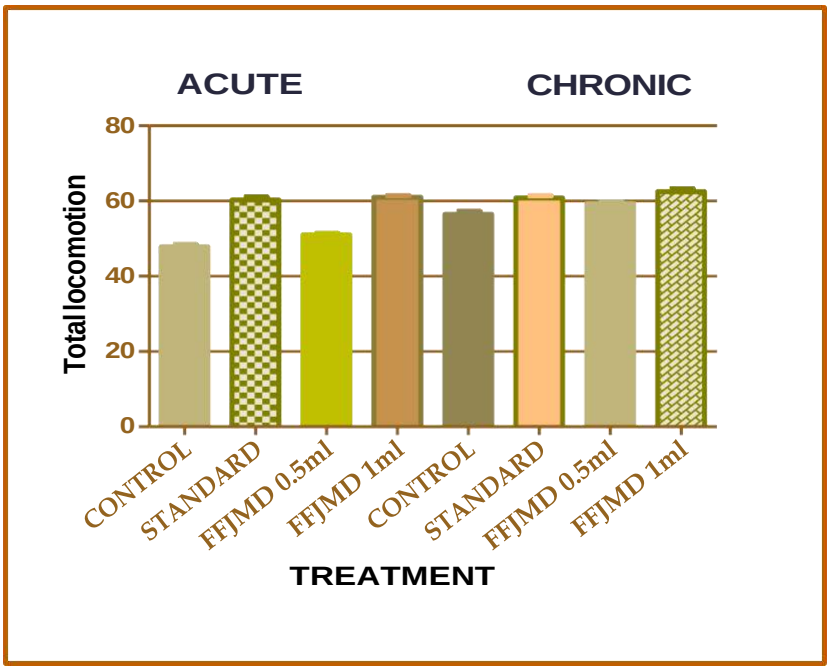


Figure 36: Effect of imipramine and FFJMD on total locomotion period of mice in acute and chronic hole board tests

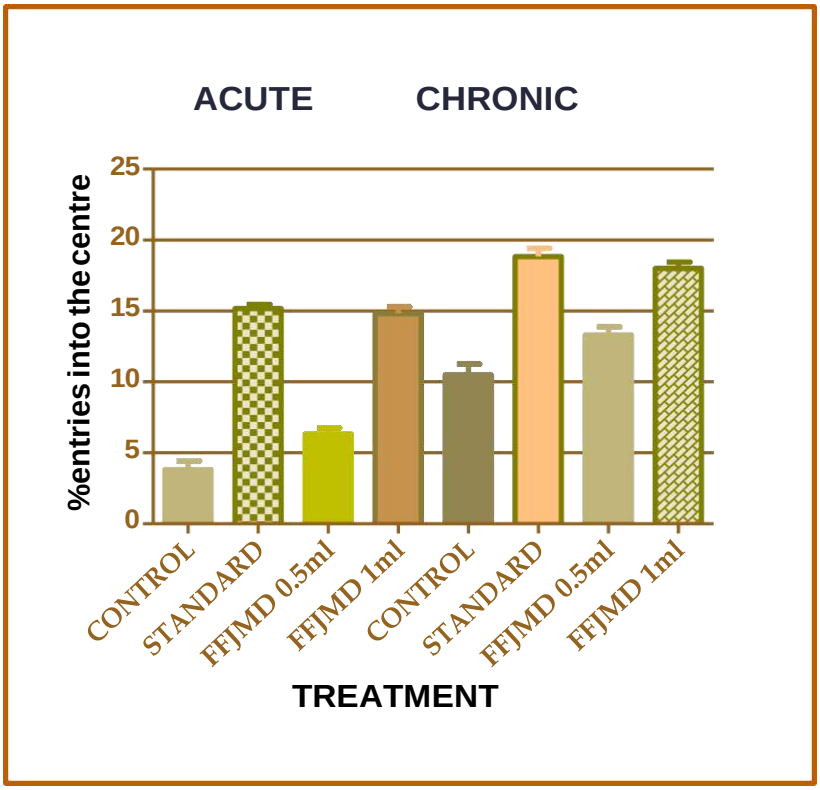


Figure 37: Effect of imipramine and FFJMD on % entries into the centre period of mice in acute and chronic hole board tests

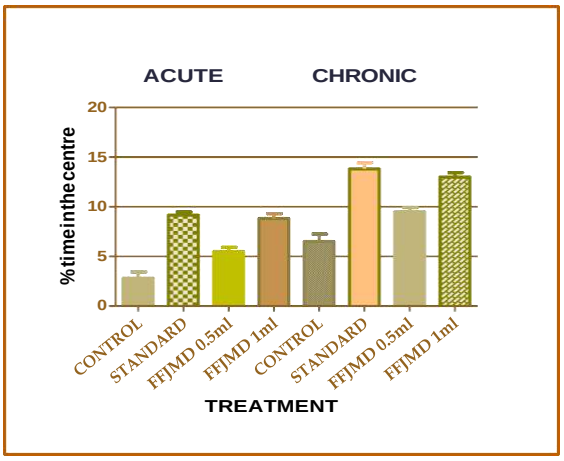


Figure 38: Effect of imipramine and FFJMD on % time in the centre period of mice in acute and chronic hole board tests

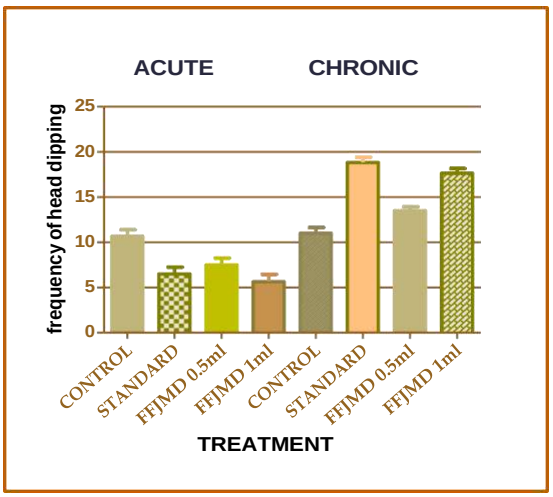


Figure 39: Effect of imipramine and FFJMD on frequency of head dipping period of mice in acute and chronic hole board tests

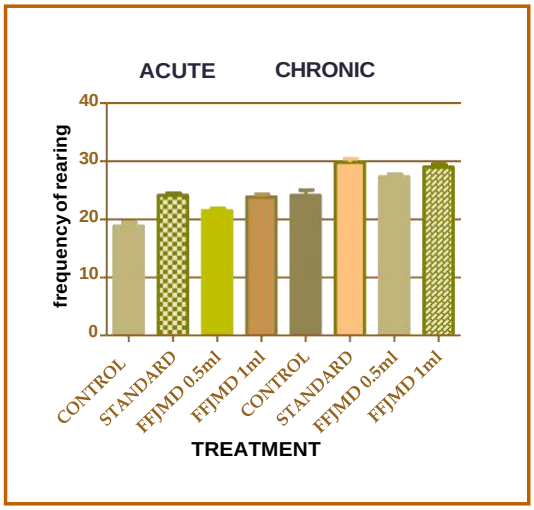


Figure 40: Effect of imipramine and FFJMD on frequency of rearing of mice in acute and chronic hole board tests

Chapter 6

Discussion

DISCUSSION

Rational behind topic investigated:

There has been an alarming increase in the psychosomatic diseases of which depression and anxiety are the leading cause of morbidity. Depression is an extremely common psychiatric condition. It is the most common mood affective disorder which refers to a pathological change in mood state; it is varies from mild to severe and accompanied by hallucinations and delusions.[196]

According to world health organization (WHO), depression is the fourth leading cause of disease worldwide. It affects the individual‟s socioeconomic status, family life, and, therefore, need medical attention.

Many drugs are available for the treatment of depression, including tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), serotonin-noradrenergic reuptake inhibitors (SNRIs) and monoamine oxidase inhibitors (MAOIs).[197]

Historically monoamine oxidase inhibitors were used as first-line treatment of psychiatric and neurological disorders. Significant progress in antidepressant therapy has been substantially made to manage the depression over the past half century but emerging data have questioned the safety and efficacy of these drugs.[198]

Recently the new generations of antidepressants with high degrees of selectivity for MAO inhibitors have become the most widely prescribed drugs in clinical application.

Pharmacological screening of test compounds:

Acute and chronic stress is used in animal models to induce behavioral, physiological and neural changes relative to human depression.[199]

The present study showed that oral treatment of mice with the test compounds FFJMD did not induce mortality or significant clinical symptoms of toxicity.

In the present study antidepressants like potentials of test compounds were investigated in two widely accepted and most commonly used behavioral models viz. tail suspension test and forced swim test model in rodents.

Porsolt *et al.,* (1978)[200] described an animal model for assessing the effect of

antidepressant drugs. The animal model was based on behavioral despair, i.e. the mice after placing in water become immobile and float with stretched limbs which are an indication of depression. Drugs which reduced the period of immobility belonged to the group of antidepressants. The Porsolt test is an extensively used, validated model and included in the battery of test for screening drugs having antidepressant-like activity.[201]

In the present study vehicle control mice remained immobile for about 2 min and in both imipramine and FFJMD treated mice showed a reduction in the period of immobility which significantly indicated antidepressant activity. Imipramine is clinically used as the antidepressant drug. Reduction in immobility time by FFJMD (0.5ml and 1ml) was identical with that exhibited by imipramine (10 mg/kg).

The first neurochemical theory of depression was monoamine hypothesis which postulated that the major cause of depression is the decrease or deficiency of biogenic amine function i.e. imbalance of available monoamines- dopamine, serotonin and norepinephrine (DA, 5HT and NE) as a result of disturbed synthesis, storage and release of monoamines[202] or subnormal monoamine receptors functioning in certain regions of the brain.[203] The clinically used antidepressant molecules that have been developed in the past were aimed to increase extracellular level of biogenic amines 5 HT, DA and NE within the brain either by blocking the reuptake or inhibiting monoamine oxidase which causes degradation of monoamines.[204]

The subsequent development of monoamine oxidase inhibitors was based on a similar approach namely an indirect elevation of extracellular concentration of the biogenic amines.

Monoamine oxidase is a mitochondrial enzyme (MAO) which catalyzes the oxidative deamination of a variety of monoamines such as serotonin, dopamine, and norepinephrine. The pathophysiology of depression involves the abnormal activity of the enzyme which leads to dysfunction in monoaminergic neurotransmission in central nervous system.[205] MAO Inhibitor is one of the important classes of antidepressants which act by inhibiting monoamine oxidase and leads to increase the neuronal monoamine level produces antidepressant activity.

Researchers reported different methods for the estimation of monoamine oxidase inhibitory activity like manometric, microfluorimetric, fluorimetric, polarographic assay and radioactive tracer techniques. The oxygen consumption may be determined using an oxygen electrode during oxidative deamination of substrates by the enzyme in the polarographic assay.[206] A simple and sensitive spectrophotometric method for determination of monoamine oxidase activity was used by many researchers to study the MAO inhibitory activity.[207]

MAO enzyme is present in two isoform MAOA and MAOB, which have been distinguished based on relative substrate specificity. Monoamine oxidase A is more specific for epinephrine, norepinephrine, and 5-hydroxytryptamine whereas monoamine oxidase B is more specific for phenylethanolamine and benzylamine. Dopamine and tyramine are handled equally well by both isoenzymes.[208] Some MAO-A inhibitors are effective for treating depression.[209] Many targets are reported for the treatment of depression like inhibition of serotonin, norepinephrine reuptake. The important target for treatment of depression is monoamine oxidase inhibitor, inhibition of which produces antidepressant activity. Inhibition of selective MAO-A leads to increase in serotonin, norepinephrine, and dopamine level in the brain.[210]

The tail suspension test is a well-characterized behavioral model that thinks to be predictive of antidepressant activity in human. It is an extensively used, validated model for screening antidepressant-like activity of drugs.

In the tail suspension test (TST), animals subjected to inescapable, aversive situation showed agitation and immobility called searching–waiting strategies of Steru *et al.* (1985) is an indication of depression.[211]

In the present study, antidepressant-like activity (duration of immobility) of newly synthesized test compounds FFJMD was studied by using forced swimming test and tail suspension test models.

The standard drug imipramine (tricyclic antidepressant) which was administered for 10 successive days showed significant ($p<0.001$) decrease in the duration of immobility in tail suspension and forced swimming test.

FFJMD at a dose of (0.5ml and 1ml) showed identical effect exhibited by standard drugs.

The monoamines play a very important role in the etiopathogenesis of depression. The spontaneous and experimentally induced deficiencies in monoamines (serotonin, norepinephrine, and dopamine) are well documented and implicated in the onset of depression. Many experimental procedures designed to increase monoaminergic activity proved antidepressant properties.[212]

The purpose of this study was assessed the anti-depressant like effect of *Malus* domestica fruit juice using animal behavioral models. A major problem in the screening for new anti- depressant effect is the establishment of a valid animal model able to sufficiently and accurately identified diverse depressant treatments, without making error of omission.

In the case, the forced swimming test, tail suspension tests, and hole board tests are widely accepted behavioral models for the assessment of anti- depressant activity. The characteristic behavioral evaluated in these tests, termed immobility, has been considered to reflect behavioral despair similar to that seen in human depression, and it is well known that anti-depressant drugs are able to reduce the immobility time in rodents.

It is interesting to note that the immobility shown by mice when subjected to un avoidable stress such as forced swimming testis thought to reflect a state of despair or lowered mood, which is thought to reflect depressive disorders in humans. There is a significant correlation between the clinical efficacy of anti-depressant drugs and their potency in the FST; this was not found in any other model. Interestingly, our data indicate that higher doses of fresh fruit juice were more effective than smaller doses both in forced swimming, tail suspension and hole board tests.

Imipramine hydrochloride acts by inhibiting nor-epinephrine (NE) reuptake and has been used as a standard drug in majority studies. The beneficial effect of imipramine hydrochloride in the forced swimming tests model seem to be due to

increased availability of these neurotransmitters (NE) and serotonin (5HT) at the post synaptic site following reuptake inhibition.

Initial hypothesis proposed that the main symptoms of depression are due to functional deficiency of cerebral mono-aminergic transmitters such as NE, 5HT, and Dopamine (DA) located at synapses. Some studies have also shown the adaptogenic effect of the plant extract via normalization of the various stress parameters and mono- aminergic leves which may provide a clue that the extract is bringing their possible anti- depressant like effect through restoration of normal mono aminergic neurotransmitters.

Phytochemical review showed the presence of flavonoids which have been reported to have multiple biological effects such as Central Nervous System disorders. *Malus domestica* fruit juice also revealed the flavonoids which may attribute the anti-depressant activity.

MAO inhibiting activity by the *Malus domestica* fruit juice and protection of dopamine hydrolysis was comparable to imipramine. Thus antidepressant like activity of the *Malus domestica* fruit juice might also be due to inhibition of MAO, resulting in increases in the brain levels of monoamines.

Recently, oxidative stress was linked with the pathophysiology of major depression, with significant correlations being found between the severity of depression and erythrocyte super oxide dismutase/lipoperoxidation levels. Meanwhile, treatment with anti- depressant reduces the oxidative stress related to depressive disorder. Additionally, some species has reported to the anti- depressant like properties, also possess antioxidant activity.

Data from earlier study suggest that *Malus domestica* fruit juice has anti-oxidant activity. Therefore, it is possible that the antioxidant activity of the fresh fruit

juice from *Malus domestica* fruit juice may contribute to its antidepressant like effects.

However, different kinds of the research study must needed to elucidate the mechanism of action of *Malus domestica* fruit juice in the CNS, the pattern of effects were observed in these experiments suggest the involvement of the norepinephrine neurotransmitters system on its antidepressant like effects. Depression is a neurological disorder that is widely prevalent to modern fast paced life. Stressful lifestyle facilitates the evolution of depressive disorder as the stress can influence the function of central nervous system by altering a number of neurotransmitters, endocrine and neuroendocrine systems.[213] The most lethal complication of depression is the suicidal behavior.[214]

Along with the classical theory of decrease in the neurotransmitter levels in the brain leading to the pathogenesis of clinical depression, recent studies have also shown the involvement of oxidative stress in the phenomenon.[215]

Depression is usually treated with a combination therapy and medications as well as lifestyle changes. Certain foods and ingredients have been linked to lessening depression including antioxidants. Antioxidants neutralize and reduced mental functioning. The search for a natural product with fast onset of action, wide safety margin and less wide side effects has come to attention. The effective components of herbs that have antidepressant-like effect include flavonoid, oligosaccharide, polysaccharide, alkaloid, organic acid.[216]

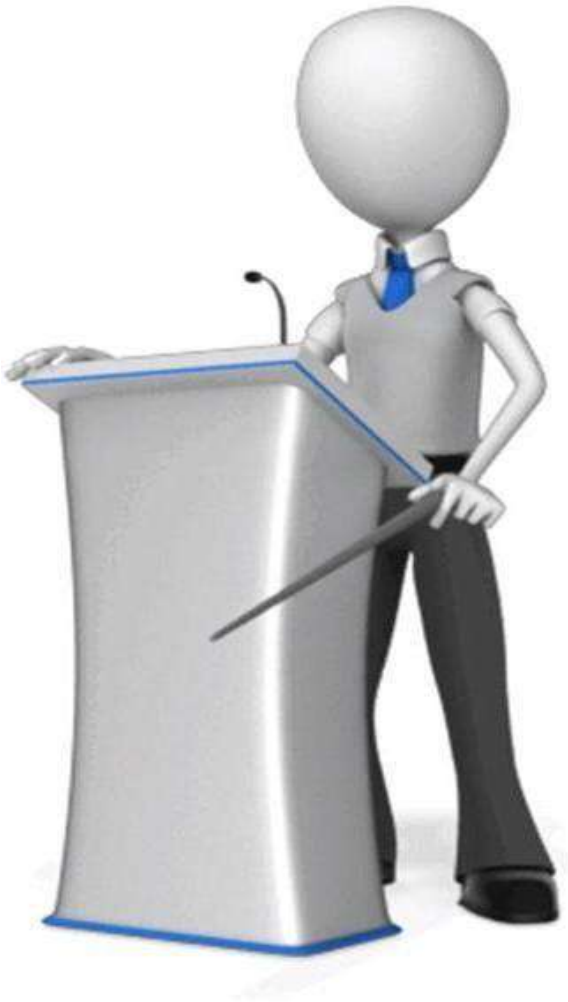
The present study was designed to elucidate the effect of juice of *Malus domestica* treating depression using Hole board Test, Forced Swim Test and Tail Suspension Test in mice.

These tests are quite sensitive and relatively specific to all major classes of antidepressant drugs.[217]

In TST, immobility reflects a state of despair which can be reduced by several agents which are therapeutically effective in human depression. Similarly, in the FST, mice are forced to swim in restricted space from which they cannot escape. HBT head dip response and latency until first entry was noted. This induces a state of behavioral despair in animals, which is claimed to reproduce a condition similar to human depression.[218] It has been reported that the TST is less stressful and has higher pharmacological sensitivity than FST and HBT.[219]

Hence the present study showed *Malus domestica* might be useful in depression, as it increase in mono amino oxidase in the brain.

Chapter 7



Summary

SUMMARY

- The fresh *Malus domestica* fruits used for the present studies were collected from Mangalore, Dakshina Kannada district Karnataka, in Dec 2018. It was authenticated by Dr. Jyothi Miranda, Associate Professor, Department of Botany, St. Aloysious College (Autonomous), Mangalore.

- The chronic FFJMD 0.5 ml and 1ml oral dose was found to produce the most significant antidepressant activity in comparison to imipramine 10mg/kg p.o.

- It is concluded that acute and chronic FFJMD 0.5ml and 1ml showed antidepressant activity when compared to imipramine 10mg/kg p.o respectively in the tail suspension test and forced swim test and hole board test in mice but more beneficial effect was found in chronic administration.

- *Malus domestica* was evaluated for anti- depressant activity by using fresh fruit juice in experimental animal models.

- The fruit of *Malus domestica* fruit were evaluated for treatment of antidepressant activity in Tail Suspension Test, Hole board test, and Forced swim test models in experimental mice.
- Juicing of the fruit and preliminary phytochemical studies of *Malus domestica* fruit revealed the presence of Flavonoids, Inositol, alkaloids, Carbohydrate, Saponins, proteins and Tannins..

- Imipramine (10mg/kg- oral) was used as standard.

- Biochemical tests had been carried out to estimate the mono amino oxidase levels in the experimental mice using the brain.

- All these observations support the findings that the juice of *Malus domestica* was able to offer significant anti-depressive in Tail suspension test, Forced swim test and the Hole board test response in experimental mice was studied.

Chapter 8

Conclusion

CONCLUSION

- The present study was undertaken to assess the antidepressant activity of *Malus domestica* fruit juice.

- The fruit juice found to have significant antidepressant activity in Tail suspension Test, Forced Swim Test, hole board test models.

- Biochemical tests have revealed that this fruit juice have comparable antidepressant activity with that of imipramine.

- The study showed that juice of *Malus domestica* significantly decreased the elevated mono amino oxidase levels in depressed mice without showing side effects.

- The preliminary phytochemical screening of the juice of *Malus domestica* fruit revealed the presence of Alkaloids, Flavonoids, inositol, proteins and Saponins. The antidepressant effect is may be due to the presence of these phyto-constituents.

- The antidepressant activity of *Malus domestica* fruit juice extract may be attributed to the individual or combined action of phyto constituents present in it.

- It is thus concluded that, acute and chronic FFJMD (0.5ml and 1ml) showed antidepressant activity similar to that of imipramine (10 mg/kg) in the forced swim, tail suspension and hole board test in mice but more beneficial effect was found in chronic administration. The mechanism of action of antidepressant activity appears to be primarily due to non-selective inhibition of brain monoamine oxidase enzyme activity.

- On observation of the biochemical parameter, and decreased mono amino oxidase levels were observed. Thus the present study showed that fruit of *Malus domestica* possesses Anti-depressant activity.

Chapter 9

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Chapter 10

Annexure

SRINIVAS COLLEGE OF PHARMACY
Valachil, Mangalore – 574 143

CERTIFICATE

This is to Certify that **Ms. Avrin Romitha Lobo & Dr. Satish S** are permitted to conduct the Animal Experimentation under Project titled **"An investigation on Anti-depressant activity of fresh fruit juice of *Malus domestica* in experimental animal models"** has been reviewed and cleared by the Institutional Animals Ethics Committee (IAEC) meeting held on 28.11.2017 at Srinivas College of Pharmacy, Valachil, Mangalore. The IAEC Approval Number for the proposal is: **SCP/IAEC/F150/P122/2017 dated 28.11.2017.**

DR. A. R. SHABARAYA
Chairman, IAEC
CHAIRMAN
Institutional Animal Ethics Committee
Srinivas College of Pharmacy
Valachil, MANGALORE - 574 143
Date: 28.11.2017

DR. PRAKASH NADOOR 28/11/2017
CPCSEA Main Nominee
Date: 28.11.2017

Note: Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by Office.

Dr Jyothi Miranda
Associate Professor & H.O.D
Department of Botany
St. Aloysius College (Autonomous)
Mangaluru -575003

AUTHENTICATION CERTIFICATE

This is to certify that the plant sample (fruits) used by Avrin Romitha Lobo, Srinivas College of Pharmacy, Valachil, Mangalore, in this project work entitled "An investigation on anti- depressant activity of fresh fruit juice of *Malus domestica* in experimental animal models'' is '***Malus domestica* Borkh.**' belongs to the Family Rosaceae.

Place: Mangaluru
Date: 21.12.2018

Dr. Jyothi Miranda
21.12.18

HEAD OF THE DEPT OF BOTANY
ST. ALOYSIUS COLLEGE
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Review Article

REVIEW ON PHARMACOLOGICAL ACTIVTIES OF *MALUS DOMESTICA*

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ABSTRACT

Malus domestica distributes around 20 countries all over the world and normally in India, Asia, Africa, North & South America, and Europe. In India it is commonly seen in Uttaranchal appear during the late summer months, Jammu and Kashmir's apple season may stretch into late November.*Malus domestica*(Family - Rosaceae) are widely consumed, rich source of phytochemicals, and epidemiological studies have linked the consumption of apple with reduced risk of some cancer, cardiovascular disease,obesity, pulmonary dysfunction, asthma and diabetes. Apple has been found to have very strong antioxidant activity, inhibit cancer cell proliferation, decrease lipid oxidation, and lower cholesterol.The paper reviews on its pharmacological activities such as antiproliferative,anti-depressant, anti-inflammatory, anti-microbial.

Keywords: *Malus domestica*, Anti-inflammatory, Anti-oxidant, Antiproliferative, Anti-depressant.

INTRODUCTION

WHO has listed over 21,000 plant species used around the world for medicinal purposes. In India, about 2500 plant species belonging to more than 1000 genera are being used in indigenous system of medicine which symbolizes the rich tradition for herb and herbal remedies.[1]From the ancient time different cultures around the world have used herbs and plants as a remedy in different diseased condition and maintain health. Many drugs prescribed today in modern medicinal system are derived from plants. Synthetic drug is known for its toxicity which sometimes needs serious medical attention. So in the recent practice of herbalism has got popularity around the globe including the developed countries due to its potency and apparent safety profile.

About 80% of the world population relies on the use of traditional medicine, which is predominantly based on plant material. Scientific studies available on medicinal plants indicate that promising phytochemicals can be developed for many health problems.[2] More over some of the pathological condition where the scientific drugs become crippled but traditional herbal therapy can be a satisfying option which demands an ample amount of research.[3] The attempt is made to present an overview of *Malusdomestica* for its phytochemical and pharmacological activities.

Malus domestica belongs to family Rosaceae, is a deciduous fruitdistributed throughout the world.*Malus domestica* generally called as Apple. Its synonyms are *Apple* (English), *Safarjan* (Gujarati), *Sev* (Hindi, Oriya), *Sebu* (Kannada),*Tsoonth*(Kashmiri),*Safar Chad* (Marathi),*Epal*(Malayalam). Apples are produced commercially in 91 countries on about 13 million acres. World apple production has increased about 17% in the last decade. Average yields are 10,000 lbs/acre worldwide.[4]

FRUIT

Fruit size typically ranges from 5-9 cm in diameter and available in different colour and it depends on various species

The centre of the fruit contains five carpels arranged in a five point star, each carpels containing 1-3 seeds.

***Malus domestica* fruit**

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CHEMICAL CONSTITUENTS

Apple contains a large concentration of flavonoids, as well as a variety of other phytochemicals, and the concentration of these phytochemicals may depend on many factors, such as cultivar of the apple, harvest and storage of the apples, and processing of the apples. Concentration of phytochemicals also varies greatly between the apple peels and the apple flesh.[5]

Apples are freshly fruit with high water content about (85%) and a low sugar content (on average, about 10-12% weight). The main carbohydrates are fructose (6%), glucose (2.2%).

The most well studied antioxidant compounds in the apples are quercetin-3 galactoside, quercetin-3-glucoside, quercetin-3-rhamnoside, catechin, epicatechin, procyanidin, cyanidin-3-galactoside, coumaric acid, chlorogenic acid, gallic acid, and phloridzin.

Recently researchers have examined the average concentrations of the major phenolic compounds in six cultivars of apples. They found that the average phenolic concentrations among the six cultivars were: quercetin glycosides, 13.2 mg/100 g fruit; vitamin C, 12.8 mg/100 g fruit; procyanidin B, 9.35 mg/100 g fruit; chlorogenic acid, 9.02 mg/100 g fruit; epicatechin, 8.65 mg/100 g fruit; and phloretin glycosides, 5.59 mg/100 g fruit.[5]

The compounds most commonly found in apple peels consist of the procyanidins, catechin, epicatechin, chlorogenic acid, phloridzin, and the quercetin conjugates. In the apple flesh, there is some catechin, procyanidin, epicatechin, and phloridzin, but these compounds are found in much lower concentrations than in the peels. Chlorogenic acid tends to be higher in the flesh than in the peel.[6]

Apple peels contain higher antioxidant compound especially quercetin, it has higher antioxidant activity and higher bioavailability then the apple flesh. Apples with the peels were also better able to inhibit cancer cell proliferation when compared to apples without the peels 65. More recent work has shown that apple peels contain two to six times (depending on the variety) more phenolic compounds than in the flesh, and two to three times more flavonoids in the peels when compared to the flesh.[7]

THERAPEUTIC USES

"An apple a day keeps doctor away". *Malus domestica* (Apple) a traditional plant widely used since Iron Age and has multiple benefits. Apart from pharmacological screening of *Malus domestica* edible properties of apples reportedly used for natural therapies as follows.[8]

- **ANTACID**
  It makes it very suitable to stop stomach acidity due to it contains pectin as well as the influence of the glycine.

- **ANTI-DIARRHEAL AND SOFT LAXATIVE**
  An apple seems contradictory its high content in pectins turn it into a good regulator of intestinal tract and absorbent value of the pectins make ideal case of colitis, diarrhea, gastroenteritis and in all those cases in that a too abundant and soft defecation is produced.

- **DIURECTIC AND DEPURATIVE**
  Apple content such as cysteine and arginine as well as the malic acid, it is very appropriate to eliminate the toxins that are stored in the body and besides fighting or preventing the diseases. It recommended in affection like uric acid, gout, urticarial and for the treatment of kidney related diseases.

- **HEARING LOSS**
  An apple vinegar has very beneficial properties to the health of the ear because it rich in potassium, magnesium zinc and manganese. One of this mineral deficiency can cause deafness.

PHARMACOLOGICAL ACTIVITIES- AN EVIDENCE BASED APPROACH

1. ANT-OXIDANT ACTIVITY

Apple, and especially its peels have been found to have a potent antioxidant activity and can greatly inhibit the growth liver cancer and colon cancer cells. The total antioxidant activity of apples with the peel was approximately 83μmol vitamin-C equivalents, which means that the antioxidant activity of 100 g apples is equivalent to about 1500 mg of vitamin-C. However, the amount of vitamin-C in 100 g of apples is only about 5.7 mg. Vitamin-C is a powerful antioxidant, but this research shows that nearly all of the antioxidant activity from apples comes from a variety of other compounds. Vitamin-C in apples contributed less than 4% of total antioxidant activity.[9]

2. ANTI-PROLIFERATIVE ACTIVITY

Apples have been shown to have potent antiproliferative activity in several studies. When Caco-2 colon cancer cells were treated with apple extracts, cell proliferation was inhibited in a dose-dependent manner

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reaching a maximum inhibition of 43% at a dose of 50 mg/mL. The same trend was seen in Hep G2 liver cancer cells with maximal inhibition reaching 57% at a dose of 50 mg/mL. Due to its unique combination of phytochemicals in the apples that are responsible for inhibiting the growth of tumor cells.[9] Apples had the third highest antiproliferative activity when compared to eleven other commonly consumed fruits. Apples without peels were significantly less effective in inhibiting Hep G2 cell proliferation when compared to apples with the peel, suggesting that apple peels possess significant antiproliferative activity. It was concluded that apple peels alone inhibited Hep G2 cell proliferation significantly more than whole apples.[10]

3. ANTI-MICROBIAL ACTIVITY

Effect of water and alcohol extracts of *M.domestica* fruit was found to be most effective against gram +ve and gram –ve bacteria such as B.subtilis, S.aureus and E.coli, P.aeuroginosa respectively.[11] According to literature, the antimicrobial activity could be influenced by the phenolic compounds and their polyphenol extracts had stronger inhibition effects on the bacteria. An *in vivo* assay is necessary to confirm the antimicrobial activities of *Malus domestica*, which could be usefully applied to the food, pharmaceuticals, and cosmetics industries. Isolation of the gene responsible for the antimicrobial activity would be an interesting future study topic aimed at identifying the molecule generating the desirable efficacy.

4. INHIBITION OF LIPID OXIDATION

It has been found that addition of apple phenolics to human serum decreased diphenylhexatriene-labeledphosphatidylcholine (DPHPC) oxidation in a dose dependent manner. DPHPC is incorporated into low-density lipoprotein (LDL), high-density lipoprotein and very low- density lipoprotein (VLDL) fractions and is an indicator of oxidation. Apple ingestion led to a decrease in DPHPC oxidation, reflecting the apples antioxidant activity in vivo. The protective effects of apples on LDL oxidation reached its peak at three hours following apple consumption and returned to baseline levels by 24 hours. Diphenylhexatrienelabeled propionic acid (DPHPA) binds to serum albumin and is a good measure of oxidation within the aqueous phase of human serum. It was also found that consumption of apples also led to a decrease in albumin DPHPA oxidation, reaching peak activity at 3 hours.[12]

5. ANTI-DEPRESSANT ACTIVITY

Apple ripe with antioxidants and fiber. An apple a day could if eaten with the rest of these foods keep the psychiatrist away, at least for stretches of time. Like berries, apples are high in antioxidants, which can help to prevent and repair oxidation damage and inflammation on the cellular level.Apple juice consumption may increase the production in the brain of the essential neurotransmitter acetylcholine. The researchers found that including apples in your daily diet may protect neuron cells against oxidative stress-induced neurotoxicity.[13]

6. CHOLESTEROL LOWERING EFFECT

Effect of *M.domestica* supplementation serum lipids and lipoproteins level in cholesterol-fed male rat showed that supplementation of *M.domestica* reduced the amount of TC, LDL and TG and increased HDL concentration. The effect can be due to antioxidant effect of compounds constituting the food was linked probably by inhibiting lipid peroxidation and decrease production of cholesterol, LDL and triglycerides.[14]

7. ANTI-INFLAMMATORY EFFECT

Effect of anti-inflammatory action of apple polyphenol extracts prevents damage to human gastric epithelial cells in vitro and to rat gastric mucosa in vivo. It was concluded that apple extract reduces gastric erosion in indomethacin(at dose of 35 mg/kg, s.c) induced injury in rats because of Phenolic compounds have been shown to exert direct antioxidant effects acting as ROS scavengers, hydrogen donating compounds, singlet oxygen quenchers, and metal ion chelators.[15]

8. ASTHMA AND PULMONARY FUNCTION

Apple consumption has been inversely linked with asthma and has also been positively associated with general pulmonary health. It has been found that an apple and pear intake was associated with decreased risk of asthma and a decrease in bronchial hypersensitivity because of it contains high concentration of antioxidant, vitamins, phenolic acid and flavonoids which helping to calm the inflammation in the airway.[16]

9. DIABETES AND WEIGHT LOSS

An apple consumption may also be associated with a lower risk for diabetes and its peels was also associated with a decreased risk in type II diabetes due to apple contains in higher concentration of quercetin.

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A study was conducted on approximately 400 hypercholestemic but nonsmoking women were randomized to one of three supplement groups: oat cookies, apples or pears, and each subject consumed one of each supplement three times per day for twelve weeks. The participants who consumed either of the fruits had a significant weight loss after 12 weeks of 1.21 kg, whereas those consuming the oat cookies did not have a significant weight loss. Those consuming fruit also had a significantly lower blood glucose level when compared to those consuming the oat cookies.[17]

OTHER HEALTH EFFECTS

Recently it has been found that crude extracts from immature apples actually inhibited enzymatic activities of cholera toxin in a dose dependent manner. Additionally, apple extracts reduced cholera toxin induced fluid accumulation in a dose dependent manner. The apple extracts were fractionated and each fraction was tested for inhibitory action on enzymatic activities of cholera toxin. The two apple extract fractions that contained highly polymerized catechins inhibited cholera toxin catalyzed ADP-ribosylation by 95% and 98%. The fraction containing non-catechin polyphenols caused only 3.5% inhibition and the fraction containing monomeric, dimeric, and trimericcatechins caused 39% inhibition.[18]

CONCLUSION

In the present review, authors have tried to describe active constituents, therapeutic uses and pharmacological activities of *Malus domestica*.It also reveals that *Malus domestica*contains several phytoconstituents andapples contain different vitamins reportedly vitamin A, B and C. Apples have high content of organic acids like malic, citric, tartaric acid, etc. which give the fruit its acid flavour and improve its keeping qualities.

The plant has been studied for its various pharmacological activities like anti-oxidant, antiproliferative, anti-depressant, anti-inflammatory, anti-microbial, inhibition of lipid oxidation and Cholesterol-lowering effect. *Malus domestica* has a great perspective for the treatment of diseases like antacid, anti-diarrheal, soft laxative, diuretic and depurative, hearing loss etc.Further studies and investigations can be performed on the plant for its various pharmacological activities.

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An investigation on anti-depressant activity of fresh fruit juice of Malus domestica in experimental animal models

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ABSTRACT

Mental depression is one of the common chronic illnesses that affect the mood, thought, physical health and behavior of an individual. India is a rich source of medicinal plants used therapeutically to treat various disorders including depression. This study was undertaken to evaluate the anti-depressant effect of acute and chronic administration of fresh fruit juice of Malus domestica in experimental animal models. We used the fresh fruit juice extract of Malus domestica (1 ml/kg and 2 ml/kg), the standard drug used was Imipramine (10 mg/kg) and the vehicle was 1% tween 80 (1 ml/kg), orally. Four groups of animals were used and each group had six animals. In the acute study drugs/vehicles were administered 60 min prior to the experiments. In the chronic study drugs/vehicles were administered for 10 days and the last dose was given on the 10th day, 60 minutes prior to the experiment. Forced Swim Test and Tail Suspension Test were used for testing antidepressant activity and parameters estimated includes estimation of the biochemical parameter (mono amino oxidase). Data were analysed using one-way ANOVA with drug treatment as the independent factor. Post-hoc comparisons were performed using Dunnett's test. In acute and chronic forced swim test as well as tail suspension test, duration of immobility was significantly reduced in the FFJMD treated group but more beneficial effect found in chronic administration in both models and decrease in a biochemical parameter such as mono amino oxidase when compared with depressive control. The antidepressant activity of 2 ml/kg was comparable to that of Imipramine 10 mg/kg. The present study suggests that fresh fruit juice of Malus domestica has more beneficial antidepressant activity in chronic administration of 2 ml/kg. It would be advisable to encourage consumption of Malus domestica to extract in patients with depression because of its nutritional and functional properties. From the present data, it concludes that fresh fruit juice of Malus domestica possesses significant anti-depressant activity.

Keywords— *Anti-depressant activity, Imipramine, Malus domestica*

1. INTRODUCTION

Depression is a chronic illness that affects people of all ages. Although there are many effective antidepressants available today, the current armamentarium of therapy is often inadequate, with unsatisfactory results in about one-third of all subjects treated.[1] This provides impetus to the search of newer and more effective antidepressants. Limitations to the use of available synthetic drugs open a way for alternative treatments for depression.

Plants have always been a source of drugs and herbal medicines are one of the ancient therapies that have stood the test of time. *Malus domestica* is widely consumed as fresh fruit and juice. It belongs to the family Rosaceae. In India it is commonly seen in Uttaranchal appear during the late summer months, Jammu and Kashmir's apple season may stretch into late November. *Malus domestica* has valuable compounds in different parts of the plant- the fruit, peel, and leaves. Apple contains a large concentration of flavonoids, as well as a variety of other phytochemicals, and the concentration of these phytochemicals may depend on many factors, such as cultivar of the apple, harvest and storage of the apples, and processing of the apples. The concentration of phytochemicals also varies greatly between the apple peels and the apple flesh.[2]

The most well studied antioxidant compounds in the apples are quercetin-3 galactoside, quercetin-3-glucoside, quercetin-3-rhamnoside, catechin, epicatechin, procyanidin, cyanidin-3-galactoside, coumaric acid, chlorogenic acid, gallic acid, and phloridzin. Recently researchers have examined the average concentrations of the major phenolic compounds in six cultivars of apples. They found that the average phenolic concentrations among the six cultivars were: quercetin glycosides, 13.2 mg/100 g fruit; vitamin C, 12.8 mg/100 g fruit; procyanidin B, 9.35 mg/100 g fruit; chlorogenic acid, 9.02 mg/100 g fruit; epicatechin, 8.65 mg/100 g fruit; and phloretin glycosides, 5.59 mg/100 g fruit.[3] The compounds most commonly found in apple peels consist of the

procyanidins, catechin, epicatechin, chlorogenic acid, phloridzin, and the quercetin conjugates. In the apple flesh, there is some catechin, procyanidin, epicatechin, and phloridzin, but these compounds are found in much lower concentrations than in the peels. Chlorogenic acid tends to be higher in the flesh than in the peel.[4] More recent work has shown that apple peels contain two to six times (depending on the variety) more phenolic compounds than in the flesh, and two to three times more flavonoids in the peels when compared to the flesh.[5]

Several lines of evidence suggest that apples and apple products possess a wide range of biological activities which may contribute to health beneficial effects against cardiovascular disease, asthma, obesity, pulmonary dysfunction and cancer.[6] Apple extracts and components, especially oligomericprocyanidins, have been shown to influence multiple mechanisms relevant for cancer prevention in invitro studies, this includesanti-mutagenic activity, modulation of carcinogen metabolism, modulation of signal transduction pathways. Apple products have been shown to prevent skin, mammary and colon carcinogenesis in animal models.[7] Apart from those mentioned edible properties of apples reportedly used for natural therapies as follows antacid, antidiarrheal, soft laxative, Diuretic and Depurative, Hearing loss.[8] The fruit is antidepressant, astringent and laxative.[9]

The apple is also an excellent dentifrice, the mechanical action of eating a fruit serving to clean both the teeth and the gums. It is used in the prevention of Cancer, weight loss, cardiovascular disease, diabetes, asthma, alzheimer's and Parkinson, blood sugar regulation, boost immunity, anaemia, Rheumatism.[10] Different authors have studied various parts of *Malus domestica* based products such as juice, wine and jam. However, the medicinal properties of *Malus domestica* have been scantily studied. The synergistic action of the constituents of the whole fruit may be superior to that of individual constituents. The CNS activity of *Malus domestica* is a less touched field and there is no report on the antidepressant activity of *Malus domestica* as a whole fruit (peel and seed). Hence the present study was planned to explore the antidepressant activity of fresh fruit juice extract of *Malus domestica* on acute and chronic administration in mice.

2. MATERIALS AND METHODS

Adult Swiss strain albino mice weighing 25-30 grams, bred in our institutional animal house were used and were housed in clean polypropylene cages in groups of three. A 12:12 hour dark/light cycle at an ambient temperature of 24 ± 2^{O}C were followed. Food and water were available *ad libitum*. Animals were acclimatized for seven days before exposure to the behavioral experiments. Experiments were performed during the light phase of the cycle (10:00-17:00). The study was approved by the Institutional Animal Ethics Committee and was carried out in accordance with the recommendations of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

2.1 Preparation of the fruit juice

The method of juicing includes, weighing the fresh fruit, (80gm) cut into appropriate sizes and mixed using a blender for two minutes. The pulp obtained was squeezed using a muslin cloth and the juice (50ml-60ml/100g) is refrigerated and used for the anti-depressant studies. Each drug solution was freshly prepared just before administration. Drugs and vehicles were administered orally and the doses of each drug were selected on the basis of earlier findings[11].

Animals were grouped into four and each group had six animals. Group 1 received 1% tween 80 in a dose of 1 ml/kg, group 2: imipramine at a dose of 10 mg/kg, group 3 & 4 received FFJMD extract of *Malus domestica* at a dose of 1 ml/kg and 2 ml/kg per day respectively[12]. In the acute study drugs/vehicles were administered 60 min prior to the experiments whereas in the chronic study drugs/vehicles were administered daily for 10 days and the last dose was given on the 10th day, 60 min prior to the experiment. The animal models used for testing antidepressant activity were forced to swim test[13] and tail suspension test[14]. In both the models the duration of immobility was measured to evaluate the antidepressant potential of compounds and parameters estimated includes estimation of the biochemical parameter (mono amino oxidase).[15]

The data has been analyzed using one-way ANOVA with drug treatment as an independent factor. Post-hoc comparisons were performed by applying Dunnett's test. $p<0.05$ was considered statistically significant.

3. RESULT

In the acute and chronic forced swim test duration of immobility was significantly reduced in the imipramine treated and FFJMD treated groups. A more significant decrease in immobility was found in the chronic administration of FFJMD treated group (Table 1). On observation of the biochemical parameter, and decreased mono amino oxidase levels were observed (Table 2).

In the acute tail suspension test, there was a decrease in the duration of immobility in the imipramine treated and FFJMD treated groups. In the chronic tail suspension test, there was a more significant decrease in the duration of immobility in the imipramine, FFJMD 1 ml/kg and FFJMD 2 ml/kg treated groups (Table 2).

Both animal models of depression used in our experiment showed that the antidepressant effect of chronic administration of FFJMD at the dose of 2 mL/kg was comparable to that of imipramine. Thus the present study showed that fruit of *Malus domestica* possesses Anti-depressant activity.

Table 1: Forced swim test

Groups	Acute forced swim test immobility in seconds (Mean ± SD)	Chronic forced swim test immobility in seconds (Mean ± SD)
Depressive control	126.8 ± 0.60	127.8 ± 0.94
Standard Imipramine 10 mg/kg	70.17 ± 0.30***	76.83 ± 0.60***
FFJMD 1 ml/kg	123.5 ± 0.76**	124.7 ± 1.14*
FFJMD 2 ml/kg	91.83 ± 0.47***	96 ± 0.44***

All the results are expressed in term of mean ± SEM n=6 animals in each group; Statistical significance was determined by ANOVA followed by Tukey's test. $^{*}P<0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$, statistically significant compared to depressive control group.

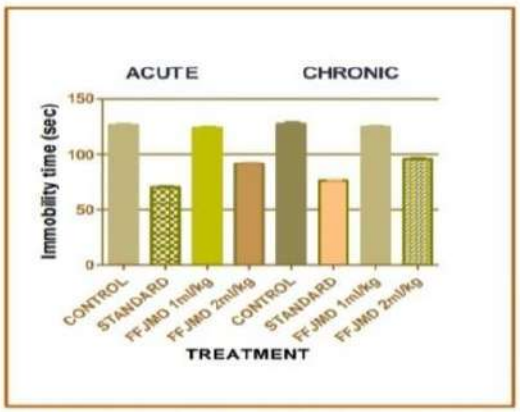

Fig. 1: Effect of imipramine and FFJMD on immobility period of mice in acute and chronic forced swim tests

Table 2: Monoamino oxidase level in the brain of mice

Dose	MAO (µg/ml)
Depressive Control	28.83±0.60
Standard Imipramine 10mg/kg	$19.17\pm0.30^{***}$
FFJMD 1ml/kg	$25.50\pm1.17^{*}$
FFJMD 2ml/kg	$15.83\pm0.47^{***}$

All the results are expressed in term of mean ± SEM n=6 animals in each group; Statistical significance was determined by ANOVA followed by Tukey's test. $^{*}P<0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$, statistically significant compared to depressive control group.

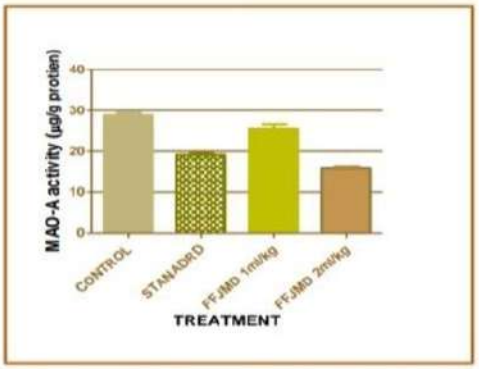

Fig. 2: Effect of *Malus domestica* fruit juice in the estimation of mono amino oxidase level in the brain of mice

Table 3: Tail suspension test

Groups	Acute tail suspension test immobility in seconds (Mean ± SD)	Chronic tail suspension test immobility in seconds (Mean ± SD)
Depressive Control	272.8 ± 0.60	289.8 ± 0.94
Standard Imipramine 10 mg/kg	$207.2 \pm 0.30^{***}$	$205.8 \pm 0.60^{***}$
FFJMD 1ml/kg	$270.5 \pm 0.76^{*}$	$293.5 \pm 0.84^{**}$
FFJMD 2 ml/kg	$222.8 \pm 0.47^{***}$	$236.0 \pm 0.44^{***}$

All the results are expressed in term of mean ± SEM n=6 animals in each group; Statistical significance was determined by ANOVA followed by Tukey's test. $^{*}P<0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$, statistically significant compared to depressive control group.

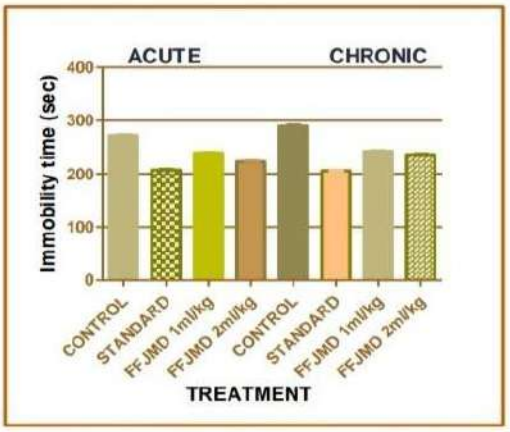

Fig. 3: Effect of imipramine and FFJMD on immobility period of mice in acute and chronic tail suspension tests

3.1 Evaluation of antidepressant activity

Fig. 4: (a) Oral route administration of drugs in mice, (b) Forced swim test in mice (mobile- immobile), (c) The brain extracted for the estimation of biochemical parameters, (d) Tail suspension test in mice (mobile- immobile)

4. DISCUSSION

In the present study, the antidepressant activity of fresh fruit juice of *Malus domestica* was studied in two classical models for screening animal models for depression, the forced swim test and tail suspension test and parameters estimated includes estimation of the biochemical parameter (mono amino oxidase).

In depression treatment is required for a prolonged period to get an optimal response; hence it is important to perform not only acute but chronic administration of the drugs in animal models. The results of the present study indicate that acute and chronic administration of fresh fruit juice extract of *Malus domestica* at a dose of 1ml/kg and 2 ml/kg has significant antidepressant activity compared to normal control but more beneficial effect was seen in chronic administration on FFJMD extracts. Decrease in a biochemical parameter such as mono amino oxidase when compared with depressive control. This antidepressant effect is comparable to that of imipramine. The present study suggests that fresh fruit juice of *Malus domestica* has antidepressant activity.

The phytochemical review showed the presence of flavonoids which have been reported to have multiple biological effects such as Central Nervous System disorders. *Malus domestica* fruit juice also revealed the flavonoids which may attribute the anti-depressant activity.

MAO inhibiting activity by the *Malus domestica* fruit juice and protection of dopamine hydrolysis was comparable to imipramine. Thus antidepressant like activity of the *Malus domestica* fruit juice might also be due to inhibition of MAO, resulting in decreases in the brain levels of monoamines.

Recently, oxidative stress was linked with the pathophysiology of major depression, with significant correlations being found between the severity of depression and erythrocyte super oxide dismutase/lipoperoxidation levels. Meanwhile, treatment with anti- depressant reduces the oxidative stress related to the depressive disorder. Additionally, some species have reported to the anti- depressant like properties, also possess antioxidant activity.

Data from the earlier study suggest that *Malus domestica* fruit juice has anti-oxidant activity. Therefore, it is possible that the antioxidant activity of the fresh fruit juice from *Malus domestica* fruit juice may contribute to its antidepressant like effects.

However, different kinds of the research study must be needed to elucidate the mechanism of action of *Malus domestica* fruit juice in the CNS, the pattern of effects was observed in these experiments suggest the involvement of the norepinephrine neurotransmitters system on its antidepressant like effects. Depression is a neurological disorder that is widely prevalent to modern fast paced life. Stressful lifestyle facilitates the evolution of depressive disorder as the stress can influence the function of the central nervous system by altering a number of neurotransmitters, endocrine and neuroendocrine systems.[16] The most lethal complication of depression is suicidal behavior.[17] Along with the classical theory of decrease in the neurotransmitter levels in the brain leading to the pathogenesis of clinical depression, recent studies have also shown the involvement of oxidative stress in the phenomenon.[18] Depression is usually treated with combination therapy and medications as well as lifestyle changes. Certain foods and ingredients have been linked to lessening depression including antioxidants. Antioxidants neutralize and reduced mental functioning. The search for a natural product with a fast onset of action, wide safety margin and less wide side effects has come to attention. The effective components of herbs that have an antidepressant-like effect include flavonoid, oligosaccharide, polysaccharide, alkaloid, organic acid.[19]

The present study was designed to elucidate the effect of the juice of *Malus domestica* treating depression using Hole board Test, Forced Swim Test and Tail Suspension Test in mice. These tests are quite sensitive and relatively specific to all major classes of antidepressant drugs.[20] In TST, immobility reflects a state of despair which can be reduced by several agents which are therapeutically effective in human depression. Similarly, in the FST, mice are forced to swim in restricted space from which they cannot escape. HBT head dip response and latency until first entry were noted. This induces a state of behavioral despair in animals, which is claimed to reproduce a condition similar to human depression.[21] It has been reported that the TST is less stressful and has higher pharmacological sensitivity than FST and HBT.[22] Hence the present study showed *Malus domestica* might be useful in depression, as it increases decrease in mono amino oxidase in the brain.

5. CONCLUSION

The present study was undertaken to assess the antidepressant activity of *Malus domestica* fruit juice. It is thus concluded that acute and chronic FFJMD (1ml/kg and 2ml/kg) showed antidepressant activity similar to that of imipramine (10 mg/kg) in the forced swim and tail suspension in mice but more beneficial effect was found in chronic administration. The mechanism of action of antidepressant activity appears to be primarily due to non-selective inhibition of brain monoamine oxidase enzyme activity. On observation of the biochemical parameter, and decreased mono amino oxidase levels were observed. Thus the present study showed that fruit of *Malus domestica* possesses Anti-depressant activity.

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An investigation on anti-depressant activity of fresh fruit juice of *Malus domestica* in experimental animal models

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Abstract

Introduction: Mental depression is one of the common chronic illnesses that affect the mood, thought, physical health and behaviour of an individual. India is a rich source of medicinal plants used therapeutically to treat various disorders including depression. This study was undertaken to evaluate the anti-depressant effect of acute and chronic administration of fresh fruit juice of *Malus domestica* in experimental animal models.

Methods: We used the fresh fruit juice extract of *Malus domestica* (1 ml/kg and 2 ml/kg), standard drug used was Imipramine (10 mg/kg) and vehicle was 1% tween 80 (1 ml/kg), orally. Four groups of animals were used and each group had six animals. In the acute study drugs/vehicles were administered 60 min prior to the experiments. In the chronic study drugs/vehicles were administered for 10 days and the last dose was given on the 10th day, 60 minutes prior to experiment. Hole Board Test was used for testing antidepressant activity and parameters estimated includes estimation of biochemical parameter (mono amino oxidase). Data was analysed using one-way ANOVA with drug treatment as the independent factor. Post-hoc comparisons were performed using Dunnett's test.

Result: In acute and chronic Hole Board Test, duration, head dipping and counts was significantly increased in the FFJMD treated group but more beneficial effect found in chronic administration in the model and decrease in biochemical parameter such as mono amino oxidase when compared with depressive control. The antidepressant activity of 2 ml/kg was comparable to that of Imipramine 10 mg/kg.

Conclusion: The present study suggests that fresh fruit juice of *Malus domestica* has more beneficial antidepressant activity in chronic administration of 2 ml/kg. It would be advisable to encourage consumption of *Malus domestica* extract in patients with depression because of its nutritional and functional properties. From the present data, it concludes that fresh fruit juice of *Malus domestica* possesses significant anti-depressant activity.

Keywords: anti-depressant activity, imipramine, *Malus domestica*

Introduction

Depression is a chronic illness that affects people of all ages. Although there are many effective antidepressants available today, the current armamentarium of therapy is often inadequate, with unsatisfactory results in about one-third of all subjects treated [1]. This provides impetus to the search of newer and more effective antidepressants. Limitations to the use of available synthetic drugs open a way for alternative treatments for depression.

Plants have always been a source of drugs and herbal medicines are one of the ancient therapies that have stood the test of time. *Malus domestica* is widely consumed as a fresh fruit and juice. It belongs to the family Rosaceae. In India it is commonly seen in Uttaranchal appear during the late summer months, Jammu and Kashmir's apple season may stretch into late November. *Malus domestica* has valuable compounds in different parts of the plant- the fruit, peel, and leaves. Apple contains a large concentration of flavonoids, as well as a variety of other phytochemicals, and the concentration of these phytochemicals may depend on many factors, such as cultivar of the apple, harvest and storage of the apples, and processing of the apples. Concentration of phytochemicals also varies greatly between the apple peels and the apple flesh [2].

The most well studied antioxidant compounds in the apples are quercetin-3 galactoside, quercetin-3-glucoside, quercetin-3-rhamnoside, catechin, epicatechin, procyanidin, cyanidin-3-galactoside, coumaric acid, chlorogenic acid, gallic acid, and phloridzin. Recently researchers have examined the average concentrations of the major phenolic compounds in six cultivars of apples. They found that the average phenolic concentrations among the six cultivars were: quercetin glycosides, 13.2 mg/100 g fruit; vitamin C, 12.8 mg/100 g fruit; procyanidin B, 9.35 mg/100 g fruit; chlorogenic acid, 9.02 mg/100 g fruit; epicatechin, 8.65 mg/100 g fruit; and phloretin glycosides, 5.59 mg/100 g fruit [3]. The compounds most commonly found in apple peels consist of the procyanidins, catechin, epicatechin, chlorogenic acid, phloridzin, and the quercetin conjugates. In the apple flesh, there is some catechin, procyanidin, epicatechin, and phloridzin, but these compounds are found in much lower concentrations than in the peels. Chlorogenic acid tends to be higher in the flesh than in the peel [4]. More recent work has shown that apple peels contain two to six times (depending on the variety) more phenolic compounds than in the flesh, and two to three times more flavonoids in the peels when compared to the flesh [5].

Several lines of evidence suggest that apples and apple products possess a wide range of biological activities which may contribute to health beneficial effects against cardiovascular disease, asthma, obesity, pulmonary dysfunction and cancer [6]. Apple extracts and components,

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The apple is also an excellent dentifrice, the mechanical action of eating a fruit serving to clean both the teeth and the gums. It is used in prevention of Cancer, weight loss, cardiovascular disease, diabetes, asthma, alzheimer's and Parkinson, blood sugar regulation, boost immunity, anaemia, Rheumatism [10]. Different authors have studied various parts of *Malus domestica* based products such as juice, wine and jam. However the medicinal properties of *Malus domestica* have been scantily studied. The synergistic action of the constituents of the whole fruit may be superior to that of individual constituents. The CNS activity of *Malus domestica* is a less touched field and there is no report on antidepressant activity of *Malus domestica* as a whole fruit (peel and seed). Hence the present study was planned to explore the antidepressant activity of fresh fruit juice extract of *Malus domestica* on acute and chronic administration in mice.

Materials and Methods

Adult Swiss strain albino mice weighing 25-30 grams, bred in our institutional animal house were used and were housed in clean polypropylene cages in groups of three. A 12:12 hour dark/light cycle at an ambient temperature of 24 ± 2oC were followed. Food and water were available *ad libitum*. Animals were acclimatized for seven days before exposure to the behavioral experiments. Experiments were performed during the light phase of the cycle (10:00-17:00). The study was approved by the Institutional Animal Ethics Committee and was carried out in accordance with the recommendations of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Preparation of the fruit juice

The method of juicing includes, weighing the fresh fruit, (80gm) cut into appropriate sizes and mixed using a blender for two minutes. The pulp obtained was squeezed using muslin cloth and the juice (50ml-60ml/100g) is refrigerated and used for the anti-depressant studies. Each drug solution was freshly prepared just before administration. Drugs and vehicles were administered orally and the doses of each drug were selected on the basis of earlier findings [11].

Animals were grouped into four and each group had six animals. Group 1 received 1% tween 80 in a dose of 1 ml/kg, group 2: imipramine at a dose of 10 mg/kg, group 3 & 4 received FFJMD extract of *Malus domestica* at a dose of 1 ml/kg and 2 ml/kg per day respectively [12]. In the acute study drugs/vehicles were administered 60 min prior to the experiments whereas in the chronic study drugs/vehicles were administered daily for 10 days and the last dose was given on the 10th day, 60 min prior to the experiment. The animal model used for testing antidepressant activity was hole board test [13]. The data on entries into a new area were used to calculate the total amount of locomotion (number of entries into all areas summed together) and the percentage of entries that were in made into the central area. The location of the animal during each of the 10-s time intervals was used to estimate the percentage of time spent in the central area.

In this model the duration, counts and head dipping was measured to evaluate the antidepressant potential of compounds and parameters estimated includes estimation of biochemical parameter (mono amino oxidase) [14].

The data has been analyzed using one-way ANOVA with drug treatment as the independent factor. Post-hoc comparisons were performed by applying Dunnett's test. $p<0.05$ was considered as statistically significant.

Fig 1: Malus domestica

Fig 2: Fresh fruit juice of Malus domestica

Result

In acute and chronic Hole Board Test, duration, head dipping and counts was significantly increased in the FFJMD treated group but more beneficial effect found in chronic administration in the model (Table 1 & 2). On observation of the biochemical parameter, and decreased mono amino oxidase levels were observed (Table 3).

The animal model of depression used in our experiment showed that the antidepressant effect of chronic administration of FFJMD at the dose of 2 mL/kg was comparable to that of imipramine. Thus the present study showed that fruit of *Malus domestica* possesses Anti-depressant activity.

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Table 1: Hole board test (Acute)

Groups	Total Locomotion	% Entries Into The Centre	% Time IN The Centre	Frequency OF Head Dipping	Frequency of Rearing
Depressive Control	47.83 ± 0.60	3.83 ± 0.60	2.83 ± 0.60	10.67 ± 0.71	18.83 ± 0.60
Standard Imipramine 10 mg/kg	60.33 ± 0.66***	15.17 ± 0.30***	9.16 ± 0.30***	6.50 ± 0.76***	24.17 ± 0.30***
FFJMD 1 ml/kg	51.00 ± 0.36**	6.33 ± 0.42**	5.50 ± 0.42**	7.50 ± 0.76*	21.50 ± 0.42**
FFJMD 2 ml/kg	61.00 ± 0.44***	14.83 ± 0.47***	8.83 ± 0.47***	5.66 ± 0.80***	23.83 ± 0.47***

All the results are expressed in term of mean±SEM n=6 animals in each group; Statistical significance was determined by ANOVA followed by Tukey's test. *P<0.05, **P< 0.01, ***P< 0.001, statistically significant compared to depressive control group.

Table 2: Hole board test (Chronic)

Groups	Total Locomotion	% Entries Into The Centre	% Time in The Centre	Frequency OF Head Dipping	frequency of Rearing
Depressive Control	56.50 ± 0.76	10.50 ± 0.76	6.50 ± 0.76	11.00 ± 0.63	24.17 ± 0.87
Standard Imipramine 10 mg/kg	60.83 ± 0.60***	18.83 ± 0.60***	13.83 ± 0.60***	18.83 ± 0.60***	29.83 ± 0.60***
FFJMD 1 ml/kg	59.33 ± 0.42*	13.33 ± 0.55**	9.50 ± 0.42**	13.50 ± 0.42*	27.33 ± 0.42**
FFJMD 2 ml/kg	62.50 ± 0.76***	18.00 ± 0.44***	13.00 ± 0.44***	17.67 ± 0.49***	29.00 ± 0.44***

All the results are expressed in term of mean±SEM n=6 animals in each group; Statistical significance was determined by ANOVA followed by Tukey's test. *P<0.05, **P< 0.01, ***P< 0.001, statistically significant compared to depressive control group.

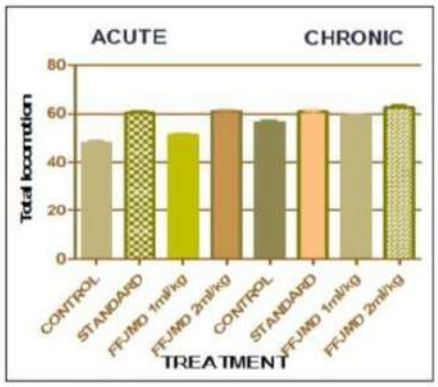

Fig 3: Effect of imipramine and FFJMD on total locomotion period of mice in acute and chronic hole board tests

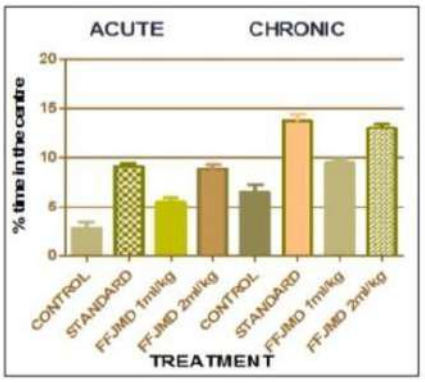

Fig 5: Effect of imipramine and FFJMD on % time in the centre period of mice in acute and chronic whole board tests

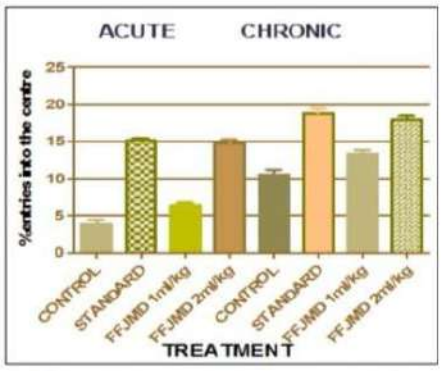

Fig 4: Effect of imipramine and FFJMD on % entries into the centre period of mice in acute and chronic hole board tests

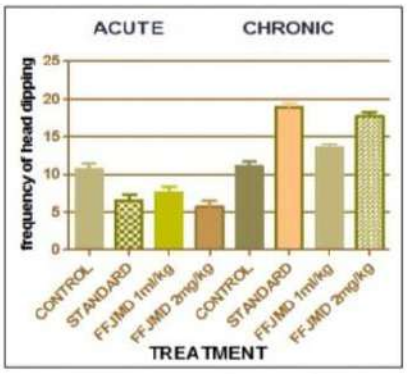

Fig 6: Effect of imipramine and FFJMD on frequency of head dipping period of mice in acute and chronic hole board tests

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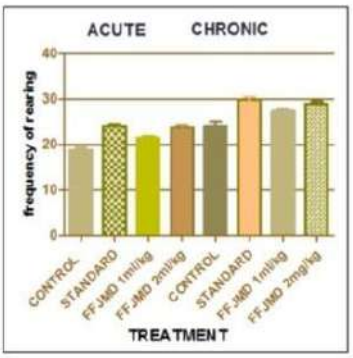

Fig 7: Effect of imipramine and FFJMD on frequency of rearing of mice in acute and chronic hole board tes

Table 3: Monoamino oxidase level in the brain of mice

Dose	MAO (µg/ml)
Depressive Control	28.83±0.60
StandardImipramine 10mg/kg	19.17±0.30***
FFJMD 1ml/kg	25.50±1.17*
FFJMD 2ml/kg	15.83±0.47***

All the results are expressed in term of mean±SEM n=6 animals in each group; Statistical significance was determined by ANOVA followed by Tukey's test. *P<0.05, **P< 0.01, ***P< 0.001, statistically significant compared to depressive control group.

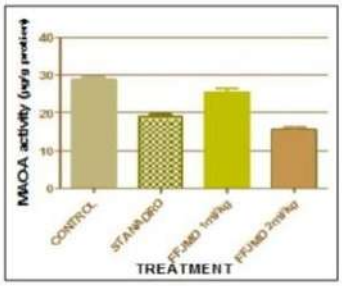

Fig 8: Effect of *Malus domestica* fruit juice in estimation of mono amino oxidase level in the brain of mice

Evaluation of antidepressant activity

A: Oral route administration of drugs in mice

B: Hole Board test in mice

C: The brain extracted for the estimation of biochemical parameters

Fig 9

Discussion

In the present study, the antidepressant activity of fresh fruit juice of *Malus domestica* were studied in classical model for screening animal model for depression, the hole board test and parameters estimated includes estimation of biochemical parameter (mono amino oxidase).

In depression treatment is required for a prolonged period to get an optimal response; hence it is important to perform not only acute but chronic administration of the drugs in animal models. The results of the present study indicate that acute and chronic administration of fresh fruit juice extract of *Malus domestica* at a dose of 1ml/kg and 2 ml/kg has significant antidepressant activity compared to normal control but more beneficial effect was seen in chronic administration on FFJMD extracts. Decrease in biochemical parameter such as mono amino oxidase when compared with depressive control. This antidepressant effect is comparable to that of imipramine. The present study suggests that fresh fruit juice of *Malus domestica* has antidepressant activity.

Phytochemical review showed the presence of flavonoids which have been reported to have multiple biological effects such as Central Nervous System disorders. *Malus domestica* fruit juice also revealed the flavonoids which may attribute the anti-depressant activity.

MAO inhibiting activity by the *Malus domestica* fruit juice and protection of dopamine hydrolysis was comparable to imipramine. Thus antidepressant like activity of the *Malus domestica* fruit juice might also be due to inhibition of MAO, resulting in decreases in the brain levels of monoamines.

Recently, oxidative stress was linked with the pathophysiology of major depression, with significant correlations being found between the severity of depression and erythrocyte super oxide dismutase/lipoperoxidation levels. Meanwhile, treatment with anti- depressant reduces the oxidative stress related to depressive disorder. Additionally, some species has reported to the anti-depressant like properties, also possess antioxidant activity.

Data from earlier study suggest that *Malus domestica* fruit juice has anti-oxidant activity. Therefore, it is possible that the antioxidant activity of the fresh fruit juice from *Malus domestica* fruit juice may contribute to its antidepressant like effects.

However, different kinds of the research study must needed to elucidate the mechanism of action of *Malus domestica* fruit juice in the CNS, the pattern of effects were observed in these experiments suggest the involvement of the norepinephrine neurotransmitters system on its antidepressant like effects. Depression is a neurological disorder that is widely prevalent to modern fast paced life. Stressful lifestyle facilitates the evolution of depressive disorder as the stress can influence the function of central nervous system by altering a number of neurotransmitters, endocrine and neuroendocrine systems [15]. The most lethal complication of depression is the suicidal behavior [16]. Along with the classical theory of decrease in the neurotransmitter levels in the brain leading to the pathogenesis of clinical depression, recent studies have also shown the involvement of oxidative stress in the phenomenon [17]. Depression is usually treated with a combination therapy and medications as well as lifestyle changes. Certain foods and ingredients have been linked to lessening depression including antioxidants. Antioxidants neutralize and reduced mental functioning. The search for a natural product with fast onset of action, wide safety margin and less wide side effects has come to attention. The effective components of herbs that have antidepressant-like effect include flavonoid, oligosaccharide, polysaccharide, alkaloid, organic acid [18].

The present study was designed to elucidate the effect of juice of *Malus domestica* treating depression using Hole board Test in mice. These tests are quite sensitive and relatively specific to all major classes of antidepressant drugs [19]. HBT head dip response and latency until first entry was noted. This induces a state of behavioral despair in animals, which is claimed to reproduce a condition similar to human depression [20]. Hence the present study showed *Malus domestica* might be useful in depression, as it increase decrease in mono amino oxidase in the brain.

Conclusion

The present study was undertaken to assess the antidepressant activity of *Malus domestica* fruit juice. It is thus concluded that, acute and chronic FFJMD (1ml/kg and 2ml/kg) showed antidepressant activity similar to that of imipramine (10 mg/kg) in the hole board in mice but more beneficial effect was found in chronic administration. The mechanism of action of antidepressant activity appears to be primarily due to non-selective inhibition of brain monoamine oxidase enzyme activity. On observation of the biochemical parameter, and decreased mono amino oxidase levels were observed. Thus the present study showed that fruit of *Malus domestica* possesses Anti-depressant activity.

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